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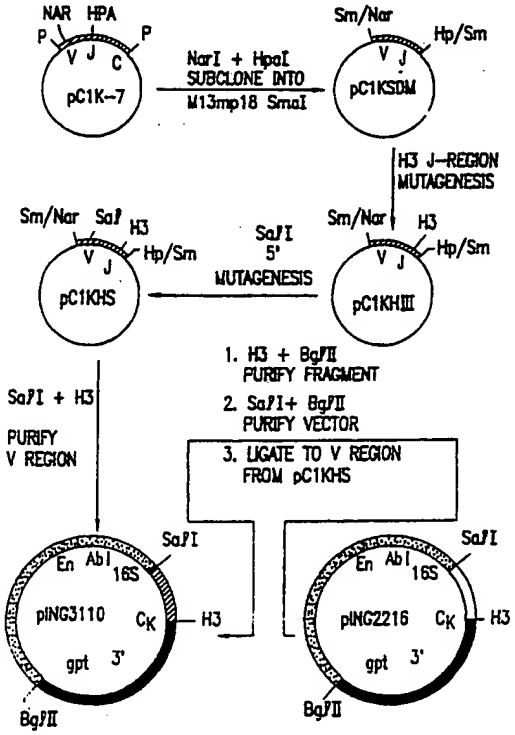
(54) Title: CHIMERIC MOUSE HUMAN ANTIBODIES WITH SPECIFICITY TO HIV ANTIGENS

(57) Abstract

Chimeric immunoglobulins, fragments or derivatives with all or a portion of a human immunoglobulin constant region and a murine variable region, having specificity for human immunodeficiency virus-1 (HIV-1) antigens, methods of their production, and their uses, have been described.



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TITLE OF THE INVENTION

CHIMERIC MOUSE HUMAN ANTIBODIES WITH SPECIFICITY TO HIV ANTIGENS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to genetically-engineered antibodies with specificity for the Human Immunodeficiency Virus (HIV) and their derivatives, nucleotide and protein sequences coding therefor, as well as methods of obtaining and manipulating such sequences.

BACKGROUND ART

Monoclonal antibody (mAb) technology has greatly impacted current thinking about human disease management. The elegant application of cell to cell fusion for the production of mAbs by Kohler and Milstein (Nature (London) 256:495, 1975) spawned a revolution in biology equal in impact to that of recombinant DNA cloning. MAbs produced from hybridomas are already widely used in diagnostic and basic scientific studies. Their efficacy in the treatment of human diseases, including viral and microbial infections, to a large extent remains to be demonstrated.

Although they display exquisite specificity and can influence the progression of human disease, mouse mAbs, by their very nature, have limitations in their applicability to human medicine. Most obviously, since they are derived from mouse cells, they are recognized as foreign protein when introduced into humans and elicit immune responses. Similarly, since they are distinguished from human proteins, they are cleared rapidly from circulation. Finally, mouse antibodies may not be recognized as effectively as human antibodies by human effector cells or molecules.

Technology to develop human mAbs that could circumvent these particular problems has met a number of obstacles. In

many cases, human mAb-producing cell lines are obtained from Epstein Barr Virus (EBV) immortalized cells derived from human blood and may therefore not be useful for scale-up and production of human pharmaceuticals. Immortalized human antibody-producing cell lines may also carry human viral sequences including the Human Immunodeficiency Virus.

In addition, since human viruses are thought to have evolved in the face of the human immune response, key antigens may not be recognized by the human immune system. Such antigens would not be expected to elicit useful immune responses in man. In contrast, those viral antigens that are immunogenic in mice can be used for the production of mouse mAbs which may have therapeutic utility in humans. The novel chimeric antibodies of this invention, have been created using both the hybridoma and genetic engineering technologies to provide reagents. The chimeric antibodies and products derived therefrom should have significant utility for the treatment and diagnosis of human disease.

SUMMARY OF THE INVENTION

The invention provides engineered chimeric mouse-human antibodies of desired variable (V) region specificity able to recognize certain antigens encoded by HIV and selected human constant (C) region properties, produced after gene cloning and expression of light (L) and heavy (H) chains. The chimeric antibodies and their derivatives may have applicability in the treatment and diagnosis of individuals infected with the Human Immunodeficiency Virus (HIV). The cloned immunoglobulin gene products and their derivatives can be produced in mammalian or microbial cells.

The invention provides cDNA sequences coding for immunoglobulin chains comprising a human C region and a non-human V region. The immunoglobulin chains are both heavy and light.

The invention provides sequences as above, present in recombinant DNA molecules, in vehicles such as plasmid vectors, capable of expression in desired prokaryotic or eukaryotic hosts.

The invention provides hosts capable of producing, by culture, the chimeric antibodies and methods of using these hosts.

The invention also provides individual chimeric immunoglobulin chains, as well as complete assembled molecules having human C regions and mouse V regions with specificity for HIV antigens, wherein both V regions have the same binding specificity.

Among other immunoglobulin chains and/or molecules provided by the invention are:

1. An antibody with monovalent specificity for an HIV antigen, i.e., a complete, functional immunoglobulin molecule comprising:
   (a) Two different chimeric H chains, one of which comprises a V region with anti-viral specificity, and
   (b) Two different L chains, with the corresponding specificities as the V regions of the H chains. The resulting heterobifunctional antibody would exhibit monovalent binding specificity toward a viral antigen.
2. Antibody fragments such as Fab, Fab', and $F(ab')_2$.

Genetic sequences, especially cDNA sequences, coding for the aforementioned combinations of chimeric chains are also provided herein.

The invention also provides for a genetic sequence, especially a cDNA sequence, coding for the V region of desired specificity of an antibody molecule H and/or L chain with or without human C regions, linked to a sequence coding for a polypeptide different than an immunoglobulin chain (e.g., an enzyme). These sequences can be assembled by the methods of

the invention, and expressed to yield mixed-function molecules.

The use of cDNA sequences is particularly advantageous over genomic sequences (which contain introns), in that cDNA sequences can be expressed in bacteria or other hosts which lack appropriate RNA splicing systems.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1. Nucleotide sequence of the coding strand for the 2E12 mouse L chain V region (V_L). Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_K2 - C_K junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 2. Nucleotide sequence of the coding strand for the 2E12 mouse H chain V region (V_H). Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_H2 - C_H1 junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 3. Construction scheme for the chimeric mouse-human Chimerl H chain mammalian expression plasmid, pING3112. The variable region from the cDNA clone pC1G-12 was engineered to be compatible with the eucaryotic expression plasmid pING2227. Plasmid pING2227 contains the following gene reguTatory elements useful for expression in mammalian cells: 1) the IgG H chain enhancer element, 2) an Abelson LTR promoter, 3) the SV40 16S splice site, and 4) the IgG H chain polyadenylation signal sequence. It also contains the entire human IgG1 C region from pGMH-6 (Liu, A. Y., et al., Proc. Natl. Acad. Sci., USA 84:3439-3443, 1987). pING3112 contains the neomycin phosphotransferase gene which allows for G418 selection in transfected cells.

FIGURE 4. Construction scheme for the chimeric mouse-human Chimerl L chain mammalian expression plasmid pING3110. The V region from the cDNA clone pC1K-7 was engineered to be compatible with the eucaryotic expression plasmid pING1712. Plasmid pING1712 contains the following gene regulatory elements useful for expression in mammalian

cells: 1) the IgH enhancer element, 2) the Abelson LTR promoter, 3) the SV40 16S splice site, and 4) a human kappa (κ) polyadenylation signal sequence. It also contains the entire human C_κ region, Liu A.Y., et al., supra, and the GPT gene which allows for mycophenolic acid resistance in transfected cells.

FIGURE 5. Construction scheme for the bacterial Chimerl Fab expression plasmid pING3127. Plasmid pING3127 contains the following elements useful for expression in E. coli: 1) the araC gene, 2) the inducible araB promoter, 3) the dicistronic Fd and K Chimerl genes fused to the pelB leader sequence, 4) the trpA transcription termination sequence, and 5) the tetR gene, useful for selection in E. coli. The specific cloning steps are described in detail in Example 3.

FIGURE 6. Yeast expression plasmids for Fab expression. Shown are the yeast expression plasmid pING3114 containing the Chimerl L chain gene fused to the yeast PGK promoter, invertase signal sequence and PGK polyadenylation signal (a); the similar yeast plasmids pING3117 and pING3137 containing the Fd gene (b); the yeast expression plasmid pING3118 containing the L chain promoter/leader fusion with PGK transcription termination signal (c); similar yeast plasmid pING3138 containing the Fd gene (d); and the final 2 gene yeast expression plasmid pING3142 (e).

FIGURE 7. Nucleotide sequence of the coding strand for the 2G12 mouse V_H region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_H3 - C_H1 junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 8. Nucleotide sequence of the coding strand for the 2G12 L chain mouse V region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the $J_\kappa1$ - C_κ junction. Also shown is the amino acid sequence deduced from

the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 9. Construction scheme for the chimeric mouse-human Chimer2 H chain mammalian expression plasmid, pING3004. The variable region from the cDNA clone pC2G-6 was engineered to be compatible with the eukaryotic expression plasmid pING2227. Plasmid pING2227 contains the gene regulatory elements described in the legend to Figure 3.

FIGURE 10. Construction scheme for the chimeric mouse-human Chimer2 L chain mammalian expression plasmid pING3005. The V region from the cDNA clone pC2K-14 was engineered to be compatible with the eucaryotic expression plasmid pING1712. Plasmid pING1712 contains the gene regulatory elements described in the legend to Figure 4.

FIGURE 11. Construction scheme for the bacterial Chimer2 Fab expression plasmid pING3211. Plasmid pING3211 contains the elements useful for expression in E. coli described in the legend to Figure 5. The specific cloning steps are described in detail in Example 5.

FIGURE 12. Nucleotide sequence of the coding strand for the 1C11 mouse V_H region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_H3 - C_H1 junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold is the oligonucleotide used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 13. Nucleotide sequence of the coding strand for the 1C11 mouse V_L region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_K1 - C_K junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold is the oligonucleotide

used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 14. Construction scheme for the chimeric mouse-human Chimer4 H chain mammalian expression plasmid, pING2255. The variable region from the cDNA clone pC4M-8 was engineered to be compatible with the eucaryotic expression plasmid pING2227. Plasmid pING2227 contains the gene regulatory elements described in the legend to Figure 3.

FIGURE 15. Construction scheme for the chimeric mouse-human Chimer4 L chain mammalian expression plasmid pING2258. The V region from the cDNA clone pC4K-16 was engineered to be compatible with the eucaryotic expression plasmid pING1712. Plasmid pING1712 contains the gene regulatory elements described in the legend to Figure 4.

FIGURE 16. Construction scheme for the bacterial Chimer4 Fab expression plasmid. This plasmid contains the elements useful for expression in E. coli described in the legend to Figure 5. The specific cloning steps are described in detail in Example 8.

FIGURE 17. Nucleotide sequence of the coding strand for the 4D12 mouse V_H region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_H4 - C_H1 junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 18. Nucleotide sequence of the coding strand for the 4D12 mouse V_L region. Shown is the nucleotide sequence from the end of the oligo-dC tail to the J_K5 - C_K junction. Also shown is the amino acid sequence deduced from the nucleotide sequence. Shown in bold are the oligonucleotides used for site directed mutagenesis and the sites at which restriction site modifications were made.

FIGURE 19. Construction scheme for the chimeric mouse-human Chimer5 H chain mammalian expression plasmid,

pING3126. The variable region from the cDNA clone pC5G-30 was engineered to be compatible with the eucaryotic expression plasmid pING2227. Plasmid pING2227 contains the gene regulatory elements described in the legend to Figure 3.

FIGURE 20. Construction scheme for the chimeric mouse-human Chimer5 L chain mammalian expression plasmid pING3132. The V region from the cDNA clone pC5K-4 was engineered to be compatible with the eucaryotic expression plasmid pING1712. Plasmid pING1712 contains the gene regulatory elements described in the legend to Figure 4.

FIGURE 21. Construction scheme for the bacterial Chimer5 Fab expression plasmid pING3139. Plasmid pING3139 contains the elements useful for expression in *E. coli* as described in the legend to Figure 5.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

GENETIC PROCESSES AND PRODUCTS

The invention provides chimeric antibodies that can be used for the treatment and diagnosis of individuals infected with HIV, either alone or in combination with other reagents. The chimeric antibodies contain mouse V regions which recognize certain antigens encoded by the HIV genome. In several of the examples described herein, the chimeric antibodies recognize those antigens recognized by the mouse mAbs 2E12, 2G12, 1C11, and 4D12.

The method of production combines five elements:

1. Isolation of messenger RNA (mRNA) from the mouse B cell hybridoma line producing the monoclonal antibody, cloning and cDNA production therefrom;
2. Preparation of a full length cDNA library from purified mRNA from which the appropriate V region gene segments of the L and H chain genes can be: (i) identified with appropriate probes, (ii) sequenced, and (iii) made compatible with a C gene segment.

3. Preparation of C region gene segment modules by cDNA preparation and cloning.
4. Construction of complete H or L chain coding sequences by linkage of the cloned specific immunoglobulin V region gene segments described in 2. above to cloned human C region gene segment modules described in 3.
5. Expression and production of chimeric L and H chains in selected hosts, including prokaryotic and eukaryotic cells.

One common feature of all immunoglobulin H and L chain genes and the encoded messenger RNAs is the so-called J region. H and L chain J regions have different sequences, but a high degree of sequence homology exists (greater than 80%) among each group, especially near the C region. This homology is exploited in this invention and consensus sequences of H and L chain J regions were used to design oligonucleotides for use as primers for introducing useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments.

C region cDNA module vectors prepared from human cells and modified by site-directed mutagenesis to place a restriction site at the analogous position in the human sequence were used. For example, one can clone the complete human C_κ region and the complete human $C_\gamma 1$ region. An alternative method utilizing genomic C region clones as the source for C region module vectors would not allow these genes to be expressed in systems such as bacteria where enzymes needed to remove intervening sequences are absent. Cloned V region segments are excised and ligated to L or H chain C region module vectors. In addition, the human $C_\gamma 1$ region can be modified by introducing a termination codon thereby generating a gene sequence which encodes the H chain portion of an Fab molecule.

The coding sequences with linked V and C regions are then transferred into appropriate expression vehicles for expression in appropriate hosts, prokaryotic or eukaryotic.

Two coding DNA sequences are said to be "operably linked" if the linkage results in a continuously translatable sequence without alteration or interruption of the triplet reading frame. A DNA coding sequence is operably linked to a gene expression element if the linkage results in the proper function of that gene expression element to result in expression of the coding sequence.

Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human C_H or C_L chain sequence having appropriate restriction sites engineered so that any V_H or V_L chain sequence with appropriate cohesive ends can be easily inserted therein. Human C_H or C_L chain sequence-containing vehicles are thus an important embodiment of the invention. These vehicles can be used as intermediates for the expression of any desired complete H or L chain in any appropriate host.

One preferred host is yeast. Yeast provides substantial advantages for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., prepeptides) (Hitzman, et al., 11th International Conference on Yeast, Genetics and Molecular Biology, Montpellier, France, September 13-17, 1982).

Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of chimeric H and L chain proteins and assembled chimeric antibodies. Any of a series of yeast gene expression systems

incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches may be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast (see Glover, D.M., ed., DNA Cloning, Vol. II, pp45-66, IRL Press, 1985).

Bacterial strains may also be utilized as hosts for the production of antibody molecules or antibody fragments described by this invention, E. coli K12 strains such as E. coli W3110 (ATCC 27325), and other enterobacteria such as Salmonella typhimurium or Serratia marcescens, and various Pseudomonas species may be used.

Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches may be taken for evaluating the expression plasmids for the production of chimeric antibodies or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, D.M., ed., DNA Cloning, Vol. I, IRL Press, 1985).

Other preferred hosts are mammalian cells, grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

Mammalian cells which may be useful as hosts for the production of antibody proteins include cells of lymphoid

origin, such as the hybridoma Sp2/O-Ag14 (ATCC CRL 1581) or the myeloma P3X63Ag8 (ATCC TIB 9), and its derivatives. Others include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61).

Many vector systems are available for the expression of cloned H and L chain genes in mammalian cells (see Glover, D.M., ed., DNA Cloning, Vol. II, pp143-238, IRL Press, 1985). Different approaches can be followed to obtain complete H_2L_2 antibodies. It is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H_2L_2 antibodies. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and L chains can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells may be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an H chain plasmid containing a second selectable marker. Cell lines producing H_2L_2 molecules via either route could be transfected with plasmids encoding additional copies of H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled H_2L_2 antibody molecules or enhanced stability of the transfected cell lines.

POLYPEPTIDE PRODUCTS

The invention provides chimeric immunoglobulin chains, either H or L, with specificity toward viral antigens of the human immunodeficiency virus, HIV. A chimeric chain contains a C region substantially similar to that present in a natural human immunoglobulin, and a non-human V region having the desired anti-viral specificity of the invention. The invention also provides immunoglobulin molecules having H and

L chains associated so that the overall molecule exhibits the desired binding and recognition properties.

Various types of immunoglobulin molecules are provided: monovalent, divalent, or molecules with the invention's V region binding domains attached to moieties carrying desired functions. This invention also provides for "fragments" of chimeric immunoglobulin molecules, which include Fab, F(ab'), and $F(ab')_2$ molecules. The invention also provides for "derivatives" of the chimeric immunoglobulins, which term includes those proteins encoded by truncated or modifed genes to yield molecular species functionally resembling the immunoglobulin fragments. The modifications include, but are not limited to, addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins and tumor necrosis factor (TNF). The fragments and derivatives can be produced from any of the hosts of this invention.

Antibodies, fragments or derivatives having chimeric H chains and L chains of the same or different V region binding specificity, can be prepared by appropriate association of the individual polypeptide chains, as taught, for example by Sears et al. (Proc. Natl. Acad. Sci. USA 72:353-357 (1975)). With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

USES

The chimeric antibodies of this invention, of which Chimer1, Chimer2, Chimer4, and Chimer5 are examples, recognize epitopes of antigens encoded by the HIV genome, including core antigens, reverse transcriptase, and envelope glycoproteins.

These HIV antigens, some of which are expressed on the surface of infected cells, include the glycoproteins encoded by the viral env gene (gp160, gp120, gp41), and proteins encoded by the viral gag gene (p55, p45, p39, p24, and p18), and pol gene (p65, p51) (Musing, M.A. et al., Nature 313:450-458 (1985)). The Chimer1, Chimer2, Chimer4, and Chimer5 antibodies recognize various of these antigens (see Examples, below) and can be used alone or in combination with antibodies that recognize other viral components.

The chimeric antibodies react with viruses, subviral particles, viral proteins, viral peptides, and HIV-infected cells expressing viral antigens and should exhibit therapeutic activity in infected individuals via normal immune and host defense mechanisms. The antibodies of this invention should be useful for treatment of asymptomatic HIV-infected individuals to prevent progression of the infectious process, as well as individuals with clinical symptoms of HIV infection (Acquired Immune Deficiency Syndrome (AIDS) and AIDS Related Complex (ARC)), by reducing viral burden and alleviating symptoms related to circulating viral antigens or virus-infected cells. Circulating HIV proteins including gp120 and p24 are thought to be associated with immunological dysfunction. Administration of Chimer1, Chimer2, Chimer4, and Chimer5, either singly or in combination therapy, should benefit individuals with low titers of anti-HIV antibodies.

Treatment of an individual infected with HIV using the antibodies, fragments or derivatives of this invention comprises parenterally admininstering a single or multiple doses of the antibody, fragment or derivative. The effective dose is a function of the individual chimeric antibody, the presence and nature of a conjugated therapeutic agent (see below), the patient and his clinical status, and can vary from about 10 ng/kg body weight to about 100 mg/kg body weight. The route of administration may include intravenous,

subcutaneous, intramuscular, intrapulmonary, intraperitoneal, intranasal, intrathecal, transdermal or other known routes.

The antibodies of this invention can be adapted for therapeutic efficacy by virtue of their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC) against HIV-infected cells. For these activities, either an endogenous source or an exogenous source of effector cells (for ADCC) or complement components (for CDC) can be utilized. Antibodies that effectively mediate ADCC or otherwise mark infected cells for destruction by host effector cells should be especially useful after acute HIV infection when relatively few host cells carry the virus.

The chimeric antibodies of this invention, their fragments, and derivatives can be used therapeutically as immunoconjugates (see for review: Dillman, R.O., Ann. Int. Med. 111:592-603 (1989)). They can be coupled to cytotoxic proteins, including, but not limited to Ricin-A, Pseudomonas toxin, Diptheria toxin, and TNF. Toxins conjugated to antibodies or other ligands, are known in the art (see, for example, Olsnes, S. et al., Immunol. Today 10:291-295 (1989)). Plant and bacterial toxins typically kill cells by disrupting the protein synthetic machinery.

The chimeric antibodies of this invention can be conjugated to additional types of therapeutic moieties including, but not limited to, radionuclides, cytotoxic agents and anti-viral drugs, to treat individuals infected with HIV. Examples of radionuclides which can be coupled to antibodies and delivered in vivo to sites of antigen include ^{212}Bi, ^{131}I, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to antibodies and subsequently used for in vivo therapy include, but are not

limited to, daunorubicin, doxorubicin, methotrexate, and Mitomycin C. Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. Antiviral drugs which may be conjugated to antibodies and subsequently used for in vivo therapy include, but are not limited to, acyclovir, azidothymidine, adenine arabinoside, dideoxyinosine, and protease inhibitors. Antiviral drugs act by interfering with virus-specific enzymes such as reverse transcriptase, with viral proteases, and with the metabolism and incorporation of nucleotides into nucleic acids. For a fuller exposition of these classes of drugs which are known in the art, and their mechanisms of action, see Goodman, A.G., et al., Goodman and Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 7th Ed., Macmillan Publishing Co., 1985).

The chimeric antibodies, fragments or derivatives of this invention may be advantageously utilized in combination with other chimeric antibodies, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

Antibodies specific for HIV-infected cells should play an active role in limiting HIV spread within an individual. Earlier intervention in the infectious process may delay or prevent the later emergence of more pathogenic HIV strains or variants within an infected individual. By controlling viral replication, the antibodies, fragments or derivatives of this invention may permit the establishment and maintenance of a longer asymptomatic course which would improve the life of infected individuals.

Interventive therapy with the chimeric antibodies of this invention may also be useful either alone or in conjunction with other anti-viral therapies to prevent accidental viral exposure from developing into symptomatic disease. Prophylactic immunization with the antibodies of this invention may effectively reduce the risk of a successful

viral infection upon challenge, much as other antibody preparations are used in the management of hepatitis and cytomegalovirus infections.

The chimeric antibodies, fragments, or derivatives of this invention, attached to a solid support, can be used to remove the virus, viral antigens, or virus-infected cells from fluids or tissue or cell extracts. In a preferred embodiment, they are used to remove HIV from blood or blood plasma products. In another preferred embodiment, the chimeric antibodies are advantageously used in extracorporeal immunoadsorbent devices, which are known in the art (see, for example, Seminars in Hematology, Vol. 26 (2 Suppl. 1) (1989)). Patient blood or other body fluid is exposed to the attached antibody, resulting in partial or complete removal of circulating HIV virions, viral antigens (free or in immune complexes), or virus-infected cells, following which the fluid is returned to the body. This immunoadsorption can be implemented in a continuous flow arrangement, with or without interposing a cell centrifugation step. See, for example, Terman, D.S. et al., J. Immunol. 117:1971-1975 (1976).

Specifically, the chimeric antibodies of this invention can be used for any and all uses in which the murine mAbs can be used, with the clear advantage that mouse-human chimeric antibodies are more compatible with the human body.

Having now generally described the invention, the same will be further understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE 1

A Chimeric Mouse-Human Immunoglobulin (Chimer1) Produced by Mammalian Cells and Specific for an HIV Envelope Protein

The mouse mAb 2E12 (described as anti-gp120,160 mAb by Yoshihara, P. et al., Proc. 4th Int'l. Conf. on AIDS, June, 1988, p237) was derived from a hybridoma fusion utilizing spleen cells from a BALB/c mouse immunized with an HIV viral lysate, and was then cloned in the presence of purified env gene product. The Sp2/0 myeloma line was used as the fusion partner. Clone 2E12 produces immunoglobulin of the IgG1 subclass. MAb 2E12 was reactive against cloned env gene product by ELISA analysis. In addition, 2E12 was capable of immunofluorescent staining of HIV-infected cells. Western blot analysis of the 2E12 mAb against viral lysates demonstrated a predominant 120 kD band as well as a weaker 160 kD band, depending upon the lysate used.

1. Recombinant Plasmid and Bacteriophage DNAs

Oligo-dG tailed pBR322, pUC18, pUC19, M13mp18, and M13mp19 were purchased from BRL (Gaithersburg, MD). DNA manipulations involving purification of plasmid DNA by buoyant density centrifugation, restriction endonuclease digestion, purification of DNA fragments by agarose gel electrophoresis, ligation and transformation of E. coli were as described by Maniatis, T., et al., Molecular Cloning: A Laboratory Manual, (1982), or other standard procedures. Restriction endonucleases and other DNA/RNA modifying enzymes were purchased from Boehringer-Mannheim (Indianapolis, IN), BRL, and New England Biolabs (Beverly, MA).

2. RNA Purification and cDNA Library Construction

One liter of 2E12 hybridoma cells at approximately 1 x 10^6 cells/ml were collected by centrifugation and washed in 100 ml of PBS (8g NaCl, 0.2g KH_2PO_4, 1.15g Na_2HPO_4,and 0.2g KCl per liter). The cells were centrifuged again, the cell pellet suspended in a solution of guanidine thiocyanate, and total cellular RNA was prepared from tissue culture cells by the method described in Maniatis, T., et al., supra. Preparation of poly(A)+ RNA was as described by Maniatis, T., et al., supra. Oligo-dT primed cDNA libraries were prepared from poly(A)+ RNA by the methods of Gubler, V., et al. (Gene 25:263 (1983)). The cDNA was dC-tailed with terminal deoxynucleotide transferase and annealed to dG-tailed pBR322. cDNA libraries were screened by hybridization (Maniatis, T., et al., supra) with ^{32}P-labeled, nick-translated DNA fragments, i.e., for κ clones with a mouse C_κ region probe and for H chain clones with a mouse IgG1 C region probe.

The V_L and V_H region fragments from the full length cDNA clones, pC1K-7 and pC1G-12 respectively, were inserted into M13 bacteriophage vectors for nucleotide sequence analysis. The complete nucleotide sequences of the V region of these clones were determined (Figures 1 and 2) by the dideoxy chain termination method. These sequences predict V region amino acid compositions that agree well with the observed compositions, and predict peptide sequences which have been verified by direct amino acid sequencing of portions of the V regions.

The nucleotide sequences of the cDNA clones show that they are immunoglobulin V region clones as they contain amino acid residues diagnostic of V domains (Kabat, et al., Sequences of Proteins of Immunological Interest, U.S. Dept. of HHS, 1983).

3. Construction of Chimeric Expression Plasmids

Expression vectors appropriate for the insertion of V_H and V_L gene modules to obtain expression of Chimer1 were constructed. The L chain vector pING1712 was made by first making a plasmid DNA containing a test chimeric L chain gene (pING2122) and adding a mouse Abelson LTR promoter, a splice region, and a mouse genomic kappa region 3' to the polyadenylation signal. The H chain mouse enhancer 0.7 kb XbaI to EcoRI fragment from M13 M8alphaRX12 (Robinson, R.R., et al., supra) was inserted into XbaI plus EcoRI cut M13mp19. The enhancer-containing HindIII to BglII fragment was inserted into the BglII to HindIII region of pSH6, an E. coli recombinant plasmid DNA that contains unique XhoI, BglII, and HindIII sites, in that order. The enhancer-containing XbaI to XhoI fragment was then inserted into the enhancer XbaI to XhoI region of pING2121b, an expression plasmid identical to pING2108b (Liu, A.Y., et al., J. Immunology 139:3521 (1987)) except that the L6 V_L region (Liu, A.Y., et al., Proc. Natl. Acad. Sci. USA 84:3439 (1987)) was used in its construction instead of the 2H7 V_L region. The resulting plasmid was pING2122.

The mouse Abelson virus LTR was obtained from pelin2 (provided by Dr. Owen Witte, UCLA). pelin2 contains the p120 viral 3' LTR (Reddy, E.P., et al., Proc. Natl. Acad. Sci. USA 80: 3623 (1983)) except that the BglII site at viral position 4623 has been modified by insertion of the EcoRI oligonucleotide linker GGATTCC. The 0.8 kb EcoRI to KpnI fragment of pelin2 containing the p120 3' LTR promoter was inserted into KpnI plus EcoRI cut pUC18. The LTR was excised as an EcoRI to SalI fragment and ligated to EcoRI plus SalI cut pING2122, resulting in a plasmid where the LTR promoter is adjacent to the L6 L chain gene (pING2126). An XhoI to SalI fragment containing SV40 16S splice donor and acceptor sites was excised from pUC12/pL1 (Robinson, R.R. et al., supra) and inserted into the SalI site of pING2126, screening for the

orientation where the splice donor was between the LTR and the L chain gene (pING2133).The polyadenylation/transcription termination region of the kappa expression vector was also modified. The first step was the HindIII digestion and religation of plasmid pING2121a, which is identical to pING2108a (Liu, A.Y., et al., supra) except that the L6 V_L was used in its construction instead of the 2H7 V_L, to form pING2121a-deltaH. The 1.1 kb BglII to BamHI fragment of mouse genomic DNA distal to the polyadenylation site (Xu, M., et al., J. Biol. Chem. 261:3838 (1986) was isolated from pS107A (provided by Dr. Randolph Wall, UCLA) and inserted into the BamHI site of pING2121a-deltaH, screening for the orientation homologous to the native gene. The 3.3 kb BglII to SstI fragment containing this modified 3' region was ligated to the 5.2 kb BglII to SstI fragment of pING2121b to form pING1703. The BglII to SalI fragment of pING1703 with the modified 3' region and chimeric kappa coding sequence was ligated to the large BglII to SalI fragment of pING2133, resulting in the 9.1 kb kappa expression vector pING1712 shown in Figure 4.

The Abelson LTR promoter was also used in the chimeric H chain expression vector pING1714. pING2111 (Robinson, R.R., et al., supra) was modified by the insertion of an AatII oligonucleotide linker at the XbaI site, followed by AatII cleavage and religation to form pING1707. The AatII to SalI fragment containing the Abelson LTR promoter was excised from pING2133 and ligated to the large AatII to SalI fragment of pING1707 to form pING1711. The H chain enhancer was deleted from pING1711 by EcoRI digestion, T4 polymerase treatment, ligation to AatII oligonucleotide linker, and cleavage and religation with AatII to form the 7.7kb expression vector pING1714.

A similar plasmid, pING2227, contains two additional regulatory elements, the IgH enhancer and the human genomic IgG polyadenylation sequence. pING2227 is identical to pING1712 in the region from BglII to SalI containing the IgH

enhancer the Abelson LTR promoter, and the 16S slice donor and acceptor sites. The human genomic IgG 3′ end sequence was ligated as an 1185 bp XmaIII DNA fragment into an XmaIII site located 6 bp past the termination codon for the H chain gene in pING1714. The 1300 bp XmaIII fragment containing the genomic gamma 3′ end was isolated from a derivative of pHG3A (Ellison, et al., Nucl. Acids Res. 10:4071 (1982)).

4. Construction of Chimerl H and L Chain Expression Plasmid

The cDNA clone containing the 2E12 H chain, pC1G-12, was adapted for mammalian expression by introducing convenient restriction endonuclease sites by site directed mutagenesis (Kramer, W., et al., Nucl. Acids Res. 12:9441) into appropriate M13 subclones, Figure 3. Oligonucleotides were synthesized on a Cyclone DNA synthesizer, New Brunswick Scientific Co., and purified by acrylamide gel electrophoresis. The J region mutagenesis primer 5′-GGCTGAGGAGACGGTGACCGTGG-3′ was used to insert a BstEII site into the M13 subclone pC1GSDM, and the oligonucleotide 5′-GAGGTCCTGTCGACTTAGTAACTGT-3′ was used to insert a SalI restriction site into pC1GBstEII upstream of the initiation codon ATG. The restriction fragment containing the 2E12 V_H region bounded by SalI and BstEII was then cloned into the expression vector pING2227.

The cDNA clone containing the 2E12 L chain, pC1K-7, was adapted for mammalian expression in a similar way, Figure 4. The J region mutagenesis primer 5′-GTTTTATTTCAAGCTTGGTCC-3′ was used to insert a HindIII site into the M13 subclone pC1KSDM, and the oligonucleotide 5′-TGAGAACTTGGTCGACAGAGTCCGGCG-3′ was used to insert a SalI restriction site into pC1KHIII upstream of the initiation codon ATG. The restriction fragment containing the 2E12 V_L region bounded by SalI and HindIII was then cloned into the expression vector pING1712.

5. Stable Transfection of Mouse Lymphoid Cells for the Production of Chimeric Antibody

The cell line Sp2/0 (American Type Culture Collection #CRL1581) was grown in Dulbecco's Modified Eagle Medium plus 4.5 g/l glucose (DMEM, Gibco) plus 10% fetal bovine serum. Media were supplemented with glutamine/penicillin/streptomycin (Irvine Scientific, Irvine, California). The electroporation method of Potter, H., et al., (Proc. Natl. Acad. Sci., USA, 81:7161 (1984)) was used. After transfection, cells were allowed to recover in complete DMEM for 24 hours, and then seeded at 1-3 x 10^4 cells per well in 96-well culture plates in the presence of selective medium. G418 (GIBCO) selection was at 0.8 mg/ml, and mycophenolic acid (Calbiochem) was at 6 μg/ml plus 0.25 mg/ml xanthine. The electroporation technique gave a transfection frequency of 1-10 x 10^{-5} for the Sp2/0 cells.

The Chimerl L chain expression plasmid pING3110 was linearized by digestion with PvuI restriction endonuclease and transfected into Sp2/0 cells, giving mycophenolic acid resistant clones which were screened for L chain synthesis. The best producer after outgrowth and subsequent subcloning, was transfected with PvuI-linearized pING3112, the expression plasmid containing the Chimerl H chain gene. After selection with G418, the clone producing the most L plus H chain, Sp2/0-3310 11E10 + 3312 5C11.4C9, secreted antibody at approximately 5 μg/ml.

6. Purification of Chimerl Antibody Secreted in Tissue Culture

Sp2/0-3110 11E10.1B2 + 3112 5C11.4C9 cells were grown in culture medium HB101 (Hana Biologics) + 1% Fetal Bovine Serum, supplemented with 10mM HEPES, 1x Glutamine-Pen-Strep (Irvine Scientific #9316). The spent medium was centrifuged at about 5,000 xg for 20 minutes. The antibody level was measured by ELISA. Approximately 23L of cell culture supernatant was

concentrated 14-fold over a S10Y30 cartridge using DC-10 concentrator (Amicon Corp). Supernatant containing about 14 mg of antibody was loaded onto a 2 ml Protein A-column (Pharmacia) in 0.15 M NaCl, 10 mM sodium phosphate buffer pH 8.4. The Chimerl antibody was eluted with a step pH gradient (pH 5.6 and 3.5). Fractions containing antibody (75% yield) were combined and concentrated 22.5-fold by ultrafiltration (YM30 membrane, stirred cell, Amicon Corp.) diluted 25-fold with PBS, reconcentrated 10-fold, diluted 10-fold with PBS, and finally reconcentrated 5-fold. The antibody was stored in 1.0 ml aliquots at -20°C.

7. Study Performed on the Chimerl Antibody

A test was performed with Chimerl to show that it retained the antigen binding characteristics of the mouse 2E12 antibody. The test demonstrated that both 2E12 and Chimerl recognized the same viral antigen. Commercially available HIV Western Strips (Dupont or equivalent) were incubated with either Chimerl (2 μg/ml) or 2E12 (10-20 μg/ml). Strips incubated with 2E12 mouse antibody were incubated with rabbit anti-mouse antibody followed by Protein A-gold followed by silver enhancement (BioRad). Strips incubated with Chimerl were incubated with Protein A-gold directly. Specific binding to a viral antigen at approximately 120 kD molecular weight was detected with each antibody.

In a second test, Chimerl and 2E12 were compared in an ELISA test. The Genetics Systems (Seattle, WA) HIV-1 ELISA kit was used as specified by the manufacturer except that the second antibody used for detection recognized either the C region of mouse (2E12) or human (Chimerl) antibody. Results showing that the antibody of this invention and 2E12 react similarly are shown in Table 1.

TABLE 1.

Antibody	Concentration	A_{450}[1]	Second Antibody Specificity
Positive Control[2]	1/400	>2.0	Human Ig
Negative Control[2]	1/400	0.12	Human Ig
Chimer1	10 µg/ml	0.41	Human Ig
	50 µg/ml	0.57	Human Ig
Chimer2	10 µg/ml	>2.0	Human Ig
	50 µg/ml	>2.0	Human Ig
Normal Mouse IgG	10 µg/ml	0.21	Mouse Ig
	50 µg/ml	0.22	Mouse Ig
2E12	10 µg/ml	0.97	Mouse Ig
	50 µg/ml	1.3	Mouse Ig
2G12	10 µg/ml	>2.0	Mouse Ig
	50 µg/ml	>2.0	Mouse Ig

[1] Absorbance measured at 450 nm.

[2] Genetic Systems Positive and Negative Human Control

EXAMPLE 2

A Chimeric Mouse-Human Fab Fragment Produced in *E. coli* and Specific for an HIV Envelope Protein

Bacteria are suited for production of chimeric antibodies expressed from mammalian cDNA since entire coding sequences can be expressed from well characterized promoters. *Escherichia coli* is one of many useful bacterial species for

production of foreign proteins since a wealth of genetic information is available for optimization of its gene expression. E. coli can be used for production of foreign proteins internally or for secretion of proteins out of the cytoplasm, where they most often accumulate in the periplasmic space (Gray, et al., Gene 39:247 (1985); Oka, et al., Proc. Natl. Acad. Sci. USA 82:7212 (1985)). Secretion from the E. coli cytoplasm has been observed for many proteins and requires a signal sequence. Proteins produced internally in bacteria are often not folded properly (Schoner, et al., BioTechnology 3:151 (1985)). Protein secreted from bacteria, however, is often folded properly and assumes native secondary and tertiary structures (Hsiung, et al., BioTechnology 4:991 (1986).

A Fab molecule consists of two nonidentical protein chains linked by a single disulfide bridge. These two chains are the intact antibody L chain and the V, J, and C_H1 portions of the antibody heavy chain, Fd. The proper cDNA clones for the Chimerl L and Fd genes have already been identified. In this example, these cDNA clones were organized into a single bacterial operon (a dicistronic message) as gene fusions to the pectate lyase (pelB) gene leader sequence from Erwinia carotovora (Lei, et al., J. Bacteriol. 169:4379 (1987) and expressed from a strong regulated promoter. The result is a system for the simultaneous expression of two protein chains in E. coli, and the secretion of immunologically active, properly assembled Fab of Chimerl antibody. The following sections detail the secretion of Chimerl Fab from E. coli.

1. Assembly of the pelB Leader Sequence Cassette

Erwinia carotovora (EC) codes for several pectate lyases (polygalacturonic acid trans-eliminase) (Lei, et al., Gene 35:63 (1985)). Three pectate lyase genes have been cloned, and the DNA sequence of these genes has been determined. When cloned into E. coli under the control of a strong promoter,

the pelB gene is expressed and large quantities of pectate lyase accumulate in the periplasmic space and culture supernatant. The pelB signal sequence functions efficiently in E. coli and was used as a secretion signal for antibody genes in this example. (Other signal sequences would also be useful for this application.) The nucleotide sequence surrounding the signal sequence of the pelB gene is published (Lei, et al., supra).

The pelB signal sequence contains a HaeIII restriction site at amino acid 22, adjacent to the signal peptidase cleavage site: ala-ala. Plasmid pSS1004 (Lei, et al., supra) containing the pelB gene in pUC8 (Vierra et al., Gene 19:259 (1982)) was digested with HaeIII and EcoRI. This DNA was ligated with an eight base pair SstI linker to SspI and EcoRI cut pBR322. The resulting plasmid contained a 300 bp fragment which included the 22 amino acid leader sequence of pelB and about 230 bp of upstream E. caratovora DNA. This plasmid, pING173, contains an insert that upon digestion with SstI and treatment with T4 DNA polymerase can be ligated directly to a DNA fragment flanked by the first amino acid of a mature coding sequence for any gene to generate a protein fusion containing a functional bacterial leader sequence in frame with the incoming gene. The SstI to EcoRI restriction fragment in pING173 was cloned into pUC18 (Yanich-Perron, et al., Gene 33:103 (1985)) to generate pRR175, which contains the pelB leader and adjacent upstream non-coding sequence (including a ribosome binding site) downstream of the lac promoter. Plasmid pING1500, derived from pRR175, contains only the region from the -48 of the pelB gene to an XhoI site downstream of the pelB leader, and includes the SstI site at the junction.

2. In Vitro Mutagenesis

Site-directed in vitro mutagenesis was performed as described by Kramer, et al., supra, to introduce a BsmI restriction site into the 2E12 L chain cDNA sequence (Figure 1) at the junction of the leader peptide and mature coding region with the oligonucleotide primer 5'-TCACAATCTCCGCATTCACTCCAGAGAT-3'.

A KpnI site was similarly introduced at the junction of the leader peptide and mature coding region of the 2E12 H chain with the oligonucleotide primer 5'-GTTGCACCTGGTACCGGACACCTGTAG-3' (Figure 2).

3. Preparation of L Chain for Bacterial Expression

The Chimerl V_L region containing a BsmI restriction site at the signal sequence processing site and a unique HindIII site in the J region of pC1KHB served as the starting point for bacterial expression. The plasmid pC1KHB was cut with BsmI, treated with T4 polymerase, and digested with HindIII (Figure 5A). The 320 bp fragment containing the V_L region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI (Figure 5B) along with a HindIII to XhoI restriction fragment containing the human C_K plus 15 nucleotides of 3' genomic DNA from pING3102 (Figure 5C). The resulting plasmid that contained a pelB::Chimerl L chain fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pING3111.

4. Preparation of Fd for Bacterial Expression

The intact Chimerl chimeric Fd gene containing a KpnI restriction site at the signal sequence processing site and a BstEII restriction site in the J region in pC1GBK served as the starting point for bacterial expression. The plasmid pC1GBK was cut with KpnI, treated with T4 polymerase, and digested with BstEII (Figure 5D). The approximately 335 bp

fragment containing the Fd V region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI (Figure 5E) along with the human CH1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D (Figure 5F). The resulting plasmid that contained a pelB::Chimer1 Fd fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pING3115.

5. Multicistronic Expression System for L Chain and Fd Gene

For production of bacterially-derived Fab, both L chain and Fd need to be produced simultaneously within the cell. Using the plasmids constructed with each of these genes separately, a series of expression vectors were constructed that contain both genes aligned so that transcription from a single promoter will specify both genes. This was done in a way that minimized the noncoding DNA between the two genes. Each gene has a ribosome binding site needed for translation initiation and the identical DNA sequence from -48 to the pelB leader::antibody gene junction.

Plasmid pING3111 was cut with SphI, treated with T4 polymerase, cut with EcoRI, and the vector fragment was purified (Figure 5G). Similarly, pING3115 was cut with XhoI, treated with T4 polymerase, cut with EcoRI and the fragment containing the Fd gene was purified (Figure 5H). These two purified DNA fragments were ligated to produce pING3116, which contained the two Chimer1 gene fusions linked in close proximity. The two-gene cistron was placed under the control of the araB promoter in pING3104. Plasmid pING3116 was cut with SphI, treated with T4 polymerase, cut with XhoI, and the fragment containing the Fd and κ genes was purified (Figure 5I). This DNA fragment was ligated to the vector fragment from pING3104 that had been cut with EcoRI, treated with T4 polymerase, and cut with XhoI (Figure 5J), generating

pING3119. The unique BstEII fragment in the Fd C region was shown by restriction analysis to be in the incorrect orientation; when the Fd gene was assembled, it assembled improperly. To correct this, pING3119 was cut with BstEII and religated to generate the final Chimerl Fab vector pING3127 (Figure 5K). This vector contains all the necessary features for expression of Chimerl Fab in E. coli.

6. Production of Chimerl Fab in Bacteria

Expression of Chimerl Fab from pING3127 in E. coli is under the inducible control of the araB promoter. Upon arabinose induction, Fab secreted into the growth medium increases more than 10-fold. The E. coli strain harboring pING3127 was cultured at 32°C in 10L of minimal medium, supplemented with 1.7% glycerol as the carbon source. Dissolved oxygen was maintained at 20% of saturation by addition of a 5X concentrate of glycerol-supplemented minimal medium. The culture was induced at OD_{600}=50 with 0.05% arabinose for greater than 16 hours in a 14L fermenter (Chemap). Fab was detected in the fermentation broth by antibody sandwich ELISA using rabbit anti-human κ chain antiserum as the solid phase agent to bind Fab, followed by detection with monoclonal anti-human Fd and goat anti-mouse IgG peroxidase conjugate.

About 7L of a culture supernatant was concentrated to 2 liters using a S10Y10 cartridge (DC10 concentrator, Amicon Corp.). The concentrate was passed through a 500g DEAE cellulose type DE52, Whatman) column pre-equilibrated with 10mM sodium phosphate at pH 8.0. Sufficient 0.2M monosodium phosphate was added to adjust pH to 6.8, and the sample was concentrated over a YM10 membrane (Stirred Cell 2000, Amicon). The sample was then diluted with sufficient water and reconcentrated to 200 ml to give a conductivity of 1.1 mS/cm. The total amount of protein was estimated by a colorimetric assay, and the sample was loaded onto a carboxymethylcellulose

type (CM52, Whatman) column at a ratio of 10 mg total protein per 1g CM52 (pre-equilibrated with 10mM sodium phosphate buffer at pH 6.8). The CM52 column was eluted with a linear gradient of increasing NaCl concentration (0-0.1N) in the same phosphate buffer. The fractions containing Fab (assessed by enzyme immunoassay) were further analyzed by SDS-PAGE and then pooled. The combined Fab fractions were concentrated over a YM10 membrane to an Fab concentration of about 1 mg/ml, and stored frozen.

The Chimer1 Fab purified from E. coli has identical molecular weight properties as other Fab molecules produced from E. coli, as assessed by SDS gel electrophoresis. The bacterially-produced Chimer1 Fab is correctly assembled as a kappa plus Fd chain dimer because of its positive reaction in the ELISA detecting molecules with both Kappa and Fd determinants, and both chains are properly folded because the Fab reacts with gp120 on Western immunoblot test strips.

7. Study Performed on Chimer1 Fab

Chimer1 Fab was tested along with 2E12 mouse antibody and Chimer1 antibody for binding to antigen fixed to commercially available HIV Western immunoblot test strips (Dupont or equivalent). Chimer1 Fab was incubated with the test strip, followed by interaction with goat anti-human Fab antibody, and then with rabbit anti-goat IgG. Detection of bound antibody was with Protein A-gold followed by silver enhancement (BioRad). Chimer1 Fab specifically recognized the same size antigen on the test strip as did Chimer1 whole antibody, specifically recognizing an antigen of about 120 kD.

EXAMPLE 3

A Chimeric Mouse-Human Fab Fragment Produced in Yeast and Specific for an HIV Envelope Protein

Yeast cells are capable of expressing and secreting foreign proteins. In this example, yeast served as the host for the production of mouse-human chimeric Fab.

1. Yeast Strains and Growth Conditions

Saccharomyces cerevisiae strain PS6 (ura3 leu2 MATa) was developed at INGENE and used as a host for yeast transformations performed as described by Ito, et al., J. Bacteriol. 153:163-168 (1983). Yeast transformants were selected on SD agar (2% glucose, 0.67% yeast nitrogen base, 2% agar) and grown in SD broth buffered with 50mM sodium succinate, pH 5.5.

2. Construction of Yeast Expression Plasmids Containing Antibody Genes

The gene sequences encoding the mature form of the V_L region of 2E12 and containing a HindIII site in the J region (as described in Example 1) and a BsmI site introduced at the signal sequence processing site was fused to the human C_κ region. Plasmid pCIKHB was cut with BsmI, treated with T4 polymerase, and cut with HindIII. The V region fragment was ligated along with the human C_L region prepared from pING3102 as a HindIII to XhoI fragment into pING1149. The plasmid pING1149 contains the gene sequence encoding the yeast invertase signal sequence (Taussig, R. et al., Nucl.Acids Res. 11:1943-1954 (1983)) under control of the yeast PGK promoter (Hitzeman, R.A., et al., Nucl. Acids Res. 10:7791-780 (1982)). The resultant plasmid pING3114 (Figure 6A) contains the PGK promoter (P), along with the invertase signal sequence (S), fused to the Chimer1 coding sequence. As the result of this fusion, the gene sequence encoding the mature form of the Chimer1 L chain was fused in frame to the gene sequence encoding the yeast invertase signal sequence. The PGK

promoter - invertase signal sequence - chimeric L chain (V,C_κ) fusion was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK polyadenylation signal (Tm) to generate pING3118 (Figure 6C). The gene sequence encoding the mature form of the V_H region of Chimer1 containing a BstEII site in the J region (as described in Example 1) and a KpnI site introduced at the signal sequence processing site from pCIGBK was fused along with the human C_H1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D into pING1149 that had been cut with PstI, treated with T4 polymerase and cut with XhoI. This generated plasmids pING3117 and pING3137. Plasmid pING3117 had the correct fusion junction between the invertase leader sequence and the mature V region coding sequence, but a BstEII fragment from the C_H1 region of pF3D was in the incorrect orientation. Plasmid pING3137 had improperly fused the invertase leader to the Chimer1 V_H region, but had the C region BstEII fragment in the correct orientation. A BamHI to PstI fragment from pING3117 containing the PGK promoter, invertase leader and a portion of the Chimer1 V region was ligated along with a PstI to XhoI fragment from pING3137 which contained the remainder of the Chimer1 V region and the J-C_H1 portion into a partial 2 micron circle (2μ) expression vector containing the PGK polyadenylation signal (Tm) to generate pING3138, Figure 6D.

A single yeast expression vector containing both the chimeric L chain and Fd chain genes and their respective expression signals was constructed from pING3118 and pING3138. This final vector, pING3142, Figure 6E, contains a portion of 2 micron circle (oriY, REP3) and the two selectable markers leu2d and ura3.

3. Yeast Secretion of Chimer1 Fab

The plasmid pING3142 was transformed into S. cerevisiae PS6 and the transformants were grown in broth under selective

conditions as described above. The culture supernatants were assayed by ELISA and contained Fab levels of approximately 900 ng/ml. This material can be purified from fermentation broth and is expected to have properties identical to those of Fab produced as described in Example 2; yeast Chimer1 Fab is expected to have the same binding specificity as Fab produced in E. coli.

EXAMPLE 4

A Chimeric Mouse-Human Immunoglobulin (Chimer2) Produced in Mammalian Cells and Specific for an HIV gag Protein

The mouse mAb 2G12 (described as anti-p24 mAb in: Yoshihara, P. et al., Proc. 4th Int'l. Conf. on AIDS, June, 1988, p237; and Marcus-Sekura, C.J. et al., Biochim. Biophys. Acta 949:213-223 (1988)) was derived from a hybridoma fusion utilizing spleen cells from a BALB/c mouse immunized with purified gag gene product. The Sp2/0 myeloma line was used as the fusion partner. Clone 2G12 produced immunoglobulin of the IgG1 subclass. MAb 2G12 was reactive against cloned gag gene product by ELISA analysis. In addition, 2G12 was capable of immunofluorescent staining HIV-infected cells. Western blot analysis of the 2G12 mAb against viral lysates demonstrated predominant bands developed at 55, 45, 39, and 24 kD.

1. RNA Purification and cDNA Library Construction

One liter of 2G12 hybridoma cells at approximately 1 x 10^6 cells/ml were collected by centrifugation and washed in 100 ml of PBS. RNA was prepared, a cDNA library was constructed in pBR322, and L and H chain cDNA clones were identified as described in Example 1.

The V_L and V_H fragments from the full length cDNA clones, pC2K-14 and pC2G-6 respectively, were inserted into M13 bacteriophage vectors for nucleotide sequence analysis. The complete nucleotide sequences of the V region of these clones were determined (Figures 7 and 8) by the dideoxy chain

termination method. These sequences predict V region amino acid compositions that agree well with the observed compositions, and predict peptide sequences which have been verified by direct amino acid sequencing of portions of the V regions.

The nucleotide sequences of the cDNA clones show that they are immunoglobulin V region clones as they contain amino acid residues diagnostic of V domains (Kabat, et al., supra).

2. Construction of Chimer2 H and L Chain Expression Plasmid

The cDNA clone containing the 2G12 H chain, pC2G-6, was adapted for mammalian expression by introducing convenient restriction endonucleases sites by convenient cloning and site directed mutagenesis (Kramer, W., et al., supra) of appropriate M13 subclones, Figure 9. Oligonucleotides were synthesized on a Cyclone DNA synthesizer, New Brunswick Scientific Co., and purified by acrylamide gel electrophoresis. An NdeI site, Figure 7, located approximately 45 bp upstream of the initiation codon ATG was used for introduction of a SalI restriction site 5′ of the antibody V region. The J region mutagenesis primer 5′-GAGACGGTGACCAGAGTCCCT-3′ was used to insert a BstEII site into the M13 subclone pSW0. The restriction fragment containing the 2G12 V_H region bounded by SalI and BstEII was then cloned into the expression vector pING2240 (pING2240 is identical to pING2227 except it contains a different antibody V region).

The cDNA clone containing the 2G12 L chain, pC2K-14, was adapted for mammalian expression in a similar way, Figure 10. The J region mutagenesis primer 5′-GTTTGATTTCAAGCTTGGTGC-3′ was used to insert a HindIII site into the M13 subclone pC2-K, and the oligonucleotide 5′-TGTCTGTGTGTCGACAGTGTGATGC-3′ was used to insert a SalI restriction site into pSW6 upstream of the initiation codon ATG. The restriction fragment containing the 2G12 V_L region bounded by SalI and HindIII in pSW7 was then cloned into the expression vector pING2216 (pING2240 is

identical to pING1712 except it contains a different antibody V region).

3. Stable Transfection of Mouse Lymphoid Cells for the Production of Chimeric Antibody

The cell line Sp2/0 (American Type Culture Collection #CRL1581) was grown, transfected, and selected as described in Example 1, above. The Chimer2 L chain expression plasmid pING3005 was linearized as described in Example 1. The best producer after outgrowth and subsequent subcloning, was transfected with PvuI-linearized pING3004, the expression plasmid containing the Chimer2 H chain gene. After selection with G418, the clone producing the most L plus H chain, Sp2/0-3005 + 3004 3E9, secreted antibody at approximately 5 μg/ml.

4. Purification of Chimer2 Antibody Secreted in Tissue Culture

Sp2/0-3005 + 3004 3E9 cells were grown in culture medium HB101 (Hana Biologics) + 6% Fetal Bovine Serum, supplemented with 10 mM HEPES, 1x Glutamine-Pen-Strep (Irvine Scientific #9316). The spent medium was centrifuged at about 14,000 xg for 20 minutes and the supernatant was filtered through a .45u Millipore nitrocellulose membrane filter and stored frozen. The antibody level was measured by ELISA. Approximately 11.3L of cell culture supernatant was concentrated 7-fold over a S10Y30 cartridge using DC-10 concentrator (Amicon corp). Supernatant containing about 50 mg of antibody was loaded onto a 2 ml Protein A-column (Pharmacia) in PBS pH 7.4 several times. The Chimer2 antibody was eluted with various pH gradients (pH 7-2) and eluted between pH 3.5-4.0. Fractions containing antibody (46% yield) were combined and concentrated 85-fold by ultrafiltration (YM30 membrane, stirred cell, Amicon Corp.) diluted 25-fold with PBS, reconcentrated

10-fold, diluted 10-fold with PBS, and finally reconcentrated 4-fold. The antibody was stored in 1.0 ml aliquots at -20°C.

5. Studies Performed on the Chimer2 Antibody

A test was performed with Chimer2 to show that it retained the antigen binding characteristics of the mouse 2G12 antibody. The test demonstrated that both antibodies recognized the same viral antigen. Commercially available HIV Western Strips (Dupont or equivalent) were incubated with either Chimer2 (2 μg/ml) or 2G12 (10-20 μg/ml). Strips incubated with 2G12 mouse antibody were incubated with rabbit anti-mouse antibody followed by Protein A-gold followed by silver enhancement (BioRad). Strips incubated with Chimer2 were incubated with Protein A-gold directly. Specific binding to a viral antigen of approximately 24 kD was detected with each antibody.

In a second test, Chimer2 and 2G12 were compared in an ELISA test, as described in Example 1. Results showing that Chimer2 and 2G12 reacted similarly are shown in Table 1.

EXAMPLE 5

A Chimeric Mouse-Human Fab Fragment Produced in *E. coli* and Specific for an HIV *gag* Protein

Bacteria are suited for production of chimeric antibodies expressed from mammalian cDNA since entire coding sequences can be expressed from well characterized promoters. The following sections detail the secretion of Chimer2 Fab from *E. coli*.

1. In Vitro Mutagenesis

Site directed *in vitro* mutagenesis was performed as described by Kramer, *et al.*, *supra*, to introduce a BsmI restriction site into the 2G12 L chain cDNA sequence (Figure 8) at the junction of the leader peptide and mature coding

region with the oligonucleotide primer 5'-ATCTGGATGTCAGCATTCGCACCTGTAAG-3'.

A BsmI site was similarly introduced at the junction of the leader peptide and mature coding region of the 2G12 H chain with the oligonucleotide primer 5'-CTGGACCTCAGCATTCACACCTGCAGT-3' (Figure 7).

2. Preparation of L Chain for Bacterial Expression

The Chimer2 V_L region containing a BsmI restriction site at the signal sequence processing site and a unique HindIII site in the J region of pSW8 served as the starting point for bacterial expression. The plasmid pSW8 was cut with BsmI, treated with T4 polymerase, and digested with HindIII (Figure 11A). The approximately 320 bp fragment containing the V_L region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI (Figure 11B) along with a HindIII to XhoI restriction fragment containing the human C_κ plus 15 nucleotides of 3' genomic DNA from p3Q2 (Figure 11C). The resulting plasmid that contained a pelB::Chimer2 L chain fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pSW10-B.

3. Preparation of Fd for Bacterial Expression

The intact Chimer2 chimeric Fd gene containing a BsmI restriction site at the signal sequence processing site and a BstEII restriction site in the J region in pSW2 served as the starting point for bacterial expression. The plasmid pSW2 was cut with BsmI, treated with T4 polymerase, and digested with BstEII (Figure 11D). The approximately 336 bp fragment containing the Fd V region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI (Figure 11E) along with the human CH1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D

(Figure 11F). The resulting plasmid that contained a pelB::Chimer2 Fd fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pSW4-B.

4. Multicistronic Expression System for L Chain and Fd Gene

For production of bacterially derived Fab, both L chain and Fd need to be produced simultaneously within the cell. Using the plasmids constructed with each of these genes separately, a series of expression vectors were constructed that contain both genes aligned so that transcription from a single promoter will specify both genes. This was done in a way that minimized the noncoding DNA between the two genes. Each gene has a ribosome binding site needed for translation initiation and the identical DNA sequence from -48 to the pelB leader::antibody gene junction.

Plasmid pSW10-B was cut with SphI, treated with T4 polymerase, cut with EcoRI, and the vector fragment was purified (Figure 11G). Similarly, pSW4-B was cut with XhoI, treated with T4 polymerase, cut with EcoRI and the fragment containing the Fd gene was purified (Figure 11H). These two purified DNA fragments were ligated to produce pMB1, which contained the two Chimer2 gene fusions linked in close proximity. The two gene cistron was placed under the control of the araB promoter in pING3107. Plasmid pMB1 was cut with SphI, treated with T4 polymerase, cut with XhoI, and the fragment containing the Fd and K genes was purified (Figure 11I). This DNA fragment was ligated to the vector fragment from pING3107 that had been cut with EcoRI, treated with T4 polymerase, and cut with XhoI (Figure 11J), generating pING3211. This vector contains all the necessary features for expression of Chimer2 Fab in E. coli.

5. Production of Chimer2 Fab in Bacteria

Expression of Chimer2 Fab from pING3211 in E. coli is under the inducible control of the araB promoter. Upon arabinose induction, Fab secreted into the growth medium increases more than 10-fold. The E. coli strain harboring pING3211 was cultured at 32°C in 10L of minimal medium, supplemented with 1.7% glycerol as the carbon source. Dissolved oxygen was maintained at 20% of saturation by adition of a 5X concentrate of glycerol-supplemented minimal medium. The culture was induced at OD_{600}=50 by addition of a 5X concentrate of glycerol-supplemented minimal medium containing 20g arabinose in 3L of concentrate. The induction solution was added over a period of about 28 hours, then 5X concentrate without minimal medium was added for about 4 hours. Fab was detected in the fermentation broth by ELISA.

About 7 liters of a culture supernatant were concentrated, analyzed, and stored as in Example 2.

The Chimer2 Fab purified from E. coli has identical molecular weight properties as other Fab molecules produced from E. coli, as assessed by SDS gel electrophoresis. The bacterially-produced Chimer2 Fab is correctly assembled as a κ plus Fd chain dimer because of its positive reaction in the enzyme immunoassays detecting molecules with both κ and Fd determinants, and because it reacts with core protein on commercially available test strips.

6. Study Performed on Chimer2 Fab

Chimer2 Fab was tested along with 2G12 mouse antibody and Chimer2 antibody for binding to antigen fixed to commercially available HIV Western test strips (Dupont or equivalent). Chimer2 Fab was incubated with the test strip, followed by interaction with goat anti-human Fab antibody, and then with rabbit anti-goat IgG. Detection of bound antibody was with Protein A-gold (BioRad) followed by silver enhancement. Chimer2 Fab specifically recognized the same size antigen on

the test strip as did Chimer2 whole antibody, specifically recognizing antigen of about 24 kD.

EXAMPLE 6

A Chimeric Mouse-Human Fab Fragment Produced in Yeast and Specific for an HIV gag Protein

Yeast cells are capable of expressing and secreting foreign proteins. In this example, yeast serve as a host for the production of mouse-human chimeric Fab. Yeast strains and growth conditions are as in Example 3.

1. Construction of Yeast Expression Plasmids Containing Antibody Genes

The gene sequences encoding the mature form of the V_L region of 2G12 and containing a HindIII site in the J region (as described in Example 4) and a BsmI site introduced at the signal sequence processing site was fused to the human C_κ region. Plasmid pSW8 was cut with BsmI, treated with T4 polymerase, and cut with HindIII. The V region fragment was ligated along with the human C_L region prepared from p3Q2 as a HindIII to XhoI fragment into pING1149. The plasmid pING1149 contains the gene sequence encoding the yeast invertase signal sequence (Taussig, R., et al., supra) under control of the yeast PGK promoter (Hitzeman, R.A., et al., supra). The resultant plasmid pSW11-Y (similar to Figure 6A) contains the PGK promoter (P) along with the invertase signal sequence (S) fused to the Chimer2 coding sequence. As the result of this fusion, the gene sequence encoding the mature form of the Chimer2 L chain was fused in frame to the gene sequence encoding the yeast invertase signal sequence. The PGK promoter - invertase signal sequence - chimeric L chain (V,C_κ) fusion was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK polyadenylation signal (Tm) to generate pSW13-Y (similar to Figure 6C). The gene sequence encoding the mature form of the V_H region of

Chimer2 containing a BstEII site in the J region (as described in Example 4) and a BsmI site introduced at the signal sequence processing site from pSW2 was fused along with the human C_H1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D into pING1149 that had been cut with Pst, treated with T4 polymerase and cut with XhoI. This generated plasmid pSW5-Y (similar to Figure 6B). The PGK promoter - invertase signal sequence - chimeric Fd chain (V,CH1) fusion was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK polyadenylation signal (Tm) to generate pSW12-Y (similar to Figure 6D).

A single yeast expression vector containing both the chimeric L chain and Fd chain genes and their respective expression signals was constructed from pSW13-Y and pSW12-Y. This final vector, pING3208 (similar to Figure 6E), contains a portion of 2 micron circle (oriY, REP3) and the two selectable markers leu2d and ura3.

2. Yeast Secretion of Chimer2 Fab

The plasmid pING3208 was transformed into S. cerevisiae PS6 and the transformants were grown in broth under selective conditions as described above. The culture supernatants were assayed by ELISA and contained Fab levels of approximately 100 ng/ml. This material can be purified from fermentation broth and is expected to have properties identical to those of Fab produced as described in Example 3; yeast Chimer2 Fab is expected to have the same binding specificity as Fab produced in E. coli.

EXAMPLE 7

A Chimeric Mouse-Human Immunoglobulin (Chimer4) Produced in Mammalian Cells and Specific for the HIV Reverse Transcriptase Protein

The mouse mAb 1C11 (developed by and commercially available from Epitope Inc., Beaverton OR) was derived from a hybridoma fusion utilizing spleen cells from a BALB/c mouse immunized with an HIV viral lysate. The Sp2/0 myeloma line was used as the fusion partner. Clone 1C11 produced immunoglobulin of the IgM subclass. MAb 1C11 was reactive with the cloned pol gene product by Western blot analysis. In addition, 1C11 was capable of immunofluorescent staining of HIV-infected cells. Western blot analysis of the 1C11 mAb against viral lysates demonstrated predominant bands developed at 51 and 65 kD.

1. RNA Purification and cDNA Library Construction

One liter of 1C11 hybridoma cells at approximately 1 x 10^6 cells/ml was collected by centrifugation and washed in 100 ml of PBS. RNA was prepared and a cDNA library was constructed in pBR322 as described in Example 1. cDNA libraries were screened by hybridization (Maniatis, T., supra) with ^{32}P-labeled, nick translated DNA fragments, i.e., for kappa clones with a mouse C_κ region probe and for H chain clones with a mouse IgM C region probe.

The V_L and V_H region fragments from the full length cDNA clones, pC4K-16 and pC4M-8 respectively, were inserted into M13 bacteriophage vectors for nucleotide sequence analysis. The nucleotide sequences of the V region of these clones were determined (Figures 12 and 13) by the dideoxy chain termination method. These sequences predict V region amino acid compositions that agree well with the observed compositions, and predict peptide sequences which have been verified by direct amino acid sequencing of portions of the V regions.

The nucleotide sequences of the cDNA clones show that they are immunoglobulin V region clones as they contain amino acid residues diagnostic of V domains (Kabat, et al., supra).

2. Construction of Chimer4 H and L Chain Expression Plasmid

The cDNA clone containing the 1C11 H chain, pC4M-8, was adapted for mammalian expression by a convenient cloning strategy. The DNA sequence of the 1C11 H chain gene is very similar to the previously cloned and chimerized anti-cancer A10 antibody gene. The region from at least 45 bp upstream of the initiation codon ATG, through the antibody leader sequence and to a BamHI restriction site in the V region at amino acids 16 and 17 contains identical DNA sequence. Previously, a SalI restriction site was introduced into the A10 H chain cDNA sequence upstream of the ATG initiation codon by site-directed mutagenesis with the primer 5'ATGTCTGTGTCGACCACTGAAGAGA-3'.

The 1C11 H chain contains the J_H3 sequence which contains a PstI site at its 3' end. The HindIII site (amino acids 3 and 4) and PstI site (J region) occurring in pC4M-8 (underlined and bold in Figure 12) were used to mobilize the the unique Chimer4 V region sequences adjacent to a SalI to HindIII DNA fragment from pTK7, which shared the 5' untranslated region, leader sequence, and the shared V region amino acid sequence. The resulting plasmid, pYZ124 contained the complete Chimer4 V_H region, leader sequence and accompanying 5' untranslated sequence. The SalI to PstI fragment from pYZ124 was cloned into the expression vector pING2227 to generate the Chimer4 H chain vector pING2255 (Figure 14).

The cDNA clone containing the 1C11 L chain, pC4K-16, was adapted for mammalian expression taking advantage of site directed mutagenesis using the polymerase chain reaction (Horton, R.M., et al., 1989, Gene 77:61). A DNA fragment was amplified, using pC4K-16 as a substrate, which contained a

SalI restriction site upstream of the initiation codon ATG and a BglII site in the J region with the oligonucleotides 5'-GGCCGTCGACTCACCTGGACATGAT-3' and 5'-AGCGCAGATCTCCAGCTTGGTGCC-3'. The DNA fragment was cut with SalI and BglII and cloned into pING2016Egpt (Robinson, R.R., et al., supra) to generate an in-frame V-J-C_K chimeric gene fusion. Subsequent cloning to generate the final L chain vector, pING2258 is as described in Figure 15.

3. Stable Transfection of Mouse Lymphoid Cells for the Production of Chimeric Antibody

The cell line Sp2/0 (American Type Culture Collection #CRL1581) was grown, transfected, and selected as described in Example 1, above.

The Chimer4 L chain expression plasmid, pING2258, and the H chain expression plasmid, pING2255, were each linearized by digestion with PvuI restriction endonuclease and co-transfected into Sp2/0 cells, giving mycophenolic acid resistant clones which were screened for L + H chain synthesis and G418 resistance. The MPA- and G418-resistant clone producing the most L + H chain, Sp2/0-2258 + 2255 11F3.1D10, was used for production of Chimer4 for testing.

4. Purification and Testing of Chimer4 Antibody Secreted in Tissue Culture

Sp2/0-2258 + 2255 11F3.1D10 cells were grown in culture medium DMEM (GIBCO) supplemented with 5% fetal bovine serum, HEPES buffer, glutamine, penicillin, and streptomycin (Irvine Scientific, Irvine, CA). Chimer4 was purified in a similar manner to that described for Chimer1 and Chimer2 in Examples 1 and 4 respectively. Purified Chimer4 antibody was tested to demonstrate that it retained the antigen binding characteristics of the mouse 1C11 antibody using commercially available HIV Western strips (Dupont or equivalent), as described above for Chimer1 and Chimer2. Chimer4 and mouse

1C11 antibodies both recognized the same HIV reverse transcriptase antigen.

EXAMPLE 8

A Chimeric Mouse-Human Fab Fragment Produced in E. coli and Specific for HIV Reverse Transcriptase Protein

Bacteria are suited for production of chimeric antibodies expressed from mammalian cDNA since entire coding sequences can be expressed from well characterized promoters. The following sections detail the secretion of Chimer4 Fab from E. coli.

1. Preparation of Fd for Bacterial Expression

Since the Chimer4 H chain V region is homologous with the A10 H chain, use was made of the pelB::A10 H chain fusion in pTK15 for construction of a pelB::Chimer4 Fd gene fusion. The V region (containing regions of sequence difference between A10 and Chimer4) were removed from pTK15 by cutting it with BamHI and XhoI and purifying the vector. Sequences of interest remaining in pTK15 include the pelB leader joined to the first 16 amino acids of the A10 first framework region (FR1). The V-J region (containing 10 bp of CH1) from pING2255 was purified as a BamHI to ApaI fragment and the CH1 fragment (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pQ16D was purified as an ApaI to XhoI fragment. A three-way ligation of these fragments generated pYZ117, Figure 16.

2. Preparation of L Chain for Bacterial Expression

The Chimer4 V_L region was adapted from pC4K-16 for bacterial expression as shown in Figure 16. Two primers were used to obtain an oligonucleotide by polymerase chain reaction, 5′-GATATCCAGATGACACAGACTACATCC-3′ and 5′-AGCGCAGATCTCCAGCTTGGTGCC-3′ which generated a DNA fragment

that was blunt-ended at one end and contained a BglII site in the J region at the other end. The DNA fragment was then cloned into pING1500 that was digested with SstI, treated with T4 polymerase and cut with BamHI, generating pING3314. Plasmid pING3314 contains the pelB leader fused in-frame with the Chimer4 V_κ-J_κ region.

3. Multicistronic Expression System for L Chain and Fd Gene

For production of bacterially derived Fab, both L chain and Fd need to be produced simultaneously within the cell. Using the plasmids constructed with each of these genes separately, a series of expression vectors were constructed that contain both genes aligned so that transcription from a single promoter will specify both genes. This was done in a way that minimized the noncoding DNA between the two genes. Each gene has a ribosome binding site needed for translation initiation and the identical DNA sequence from -48 to the pelB leader::antibody gene junction.

A two-gene vector containing the entire pelB::Fd gene and the pelB-V_κ-J_κ region was constructed by cutting pING3314 with SphI, treating it with T4 polymerase and cutting it with EcoRI and cloning into it the Fd gene from pYZ117 as an XhoI, T4 polymerase treated, EcoRI cut fragment, generating pING3315.

The Fd and the κ gene fusion from pING3315 was placed under the control of the araB promoter in pING3303. Plasmid pING3315 was cut with SphI, treated with T4 polymerase and cut with XhoI, and the fragment containing the Fd and κ genes was purified. The DNA fragment was ligated into the vector fragment of pING3303 that had been cut with PstI, treated with T4 polymerase and cut with XhoI, generating pING3316. The final two-gene expression vector, pING3405, was constructed in a three way ligation with two fragments from pING3316 [ApaI to XhoI (vector) and the ApaI to HindIII (Fd/κ) and the HindIII to XhoI fragment of pYZ125. [pYZ125 contains the V_κ

region of Chimer4 fused to the C_κ region derived from pING2016Egpt]. These steps are outlined in Figure 16.

4. Production and Testing of Chimer4 Fab in Bacteria

Chimer4 Fab is produced and purified as described in Examples 2 and 5 for Chimer1 and Chimer2 Fab. The purified Chimer4 Fab is tested for antigen binding as described in Examples 2 and 5. The Chimer4 Fab is expected to have the same binding specificity as the Fab fragment of the mouse 1C11 antibody.

EXAMPLE 9

A Chimeric Mouse-Human Fab Fragment Produced in Yeast and Specific for HIV Reverse Transcriptase Protein

Yeast cells are capable of expressing and secreting foreign proteins. In this example, yeast serve as a host for the production of mouse-human chimeric Fab. This Fab molecule is identical to the Fab produced in E. coli outlined in Example 8, except that a single amino acid change occurred at position 23 (from an isoleucine to a leucine). This was an unexpected result of DNA polymerization during site directed mutagenesis. This amino acid change is not expected to alter antigen binding ability. Yeast strains and growth conditions are as in Example 3.

1. Construction of Yeast Expression Plasmids Containing Antibody Genes

The gene sequences encoding the mature form of the V_L region of 1C11 was adapted for yeast expression as follows. Sequential site directed mutagenesis of an M13 subclone of pC4K-16 with the J region primers 5'-CATCAGCCCGTTAGATCTCCAGCTTGG-3' and the leader mature primer 5'-CATCTGGATATCTGCAGTGGTACCTTGAA-3' generated pYZ122, which contained a PstI site at the leader mature junction and a

BglII site in the J region. Plasmid pYZ122 was cut with PstI, treated with T4 polymerase and cut with HindIII (a site located in the V region; see Figure 13). The V region fragment was ligated along with the VJCK region prepared from pYZ125 (see Example 8) as a HindIII to XhoI fragment into pING1149. The plasmid pING1149 contains the gene sequence encoding the yeast invertase signal sequence (Taussig, R. et al., supra) under control of the yeast PGK promoter (Hitzeman, R.A., et al., supra).

The resultant plasmid pING3157 (similar to Figure 6A) contains the PGK promoter (P), along with the invertase signal sequence (S), fused to the Chimer4 coding sequence. As the result of this fusion, the gene sequence encoding the mature form of the Chimer4 L chain was fused in-frame to the gene sequence encoding the yeast invertase signal sequence. The PGK promoter - invertase signal sequence - chimeric L chain (V,C_K) fusion was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK poly-adenylation signal (Tm) to generate pING3158 (similar to Figure 6C).

To construct an Fd yeast vector, advantage was taken of the similarity of DNA sequence between the 1C11 H chain and the A10 antibody as discussed in Examples 7 and 8. The mammalian H chain vector, pING2225 was cut with BamHI, incubated with calf intestinal alkaline phosphatase (CIAP), and cut with ApaI. The plasmid pTK14, which contains the PGK promoter - invertase signal sequence-chimeric A10 Fd chain fusion was cut with BamHI, treated with CIAP and cut with ApaI; in a separate reaction, pTK14 was cut with BamHI. The vector fragment from the former reaction and the PGK-invertase-V-region fragment from the latter were ligated to the BamHI to ApaI V-J region fragment from pING2255 to generate pYZ116. The PGK promoter - invertase signal sequence - chimeric Fd chain (V-J-C_H1) fusion (similar to Figure 6B) was cloned into a partial 2 micron circle (2μ), ura3 yeast

expression vector containing the PGK polyadenylation signal (Tm) to generate pYZ118 (similar to Figure 6D).

A single yeast expression vector containing both the chimeric L chain and Fd chain genes and their respective expression signals was constructed from pYZ118 and pING3158. This final vector, pING3159 (similar to Figure 6E), contains a portion of 2 micron circle (oriY, REP3) and the two selectable markers leu2d and ura3.

2. Yeast Secretion of Chimer4 Fab

The plasmid pING3159 is transformed into S. cerevisiae PS6 and the transformants are grown in broth under selective conditions. The culture supernatants contain Fab. This material is purified from fermentation broth and is expected to have properties identical to those of Fab produced as described in Example 8; yeast Chimer4 Fab is expected to have the same binding specificity as Fab produced in E. coli.

EXAMPLE 10

A Chimeric Mouse-Human Immunoglobulin (Chimer5) Produced in Mammalian Cells and Specific for an HIV gag Protein

The mouse mAb 4D12 (described as anti-p18 mAb in Yoshihara, P. et al., Proc. 4th Int'l. Conf. on AIDS, June, 1988, p237) was derived from a hybridoma fusion utilizing spleen cells from a BALB/c mouse immunized with purified gag gene product. The Sp2/0 myeloma line was used as the fusion partner. Clone 4D12 produces immunoglobulin of the IgG1 subclass. MAb 4D12 is reactive against the cloned gag gene product by ELISA analysis. In addition, 4D12 is capable of immunofluorescent staining of HIV-infected cells. Western blot analysis of the 4D12 mAb against viral lysates demonstrates predominant bands developed at 55, 45, and 39 kD. An additional weak band is developed at 18 kD depending upon the lysate used.

1. RNA Purification and cDNA Library Construction

One liter of 4D12 hybridoma cells at approximately 1 x 10^6 cells/ml were collected by centrifugation and washed in 100 ml of PBS. RNA was prepared, a cDNA library was constructed in pBR322, and L and H chain cDNA clones were identified as described in Example I. The L and V_H region fragments from the full length cDNA clones, pC5K-4 and pC5G-30 respectively, were inserted into M13 bacteriophage vectors for nucleotide sequence analysis. The complete nucleotide sequences of the V region of these clones were determined (Figures 17 and 18) by the dideoxy chain termination method. These sequences predict V region amino acid compositions that agree well with the observed compositions, and predict peptide sequences which have been verified by direct amino acid sequencing of portions of the V regions.

The nucleotide sequences of the cDNA clones show that they are immunoglobulin V region clones as they contain amino acid residues diagnostic of V domains (Kabat, et al., supra).

2. Construction of Chimer5 H and L Chain Expression Plasmid

The cDNA clone containing the 4D12 H chain, pC5G-30, was adapted for mammalian expression by introducing convenient restriction endonucleases sites by convenient cloning and site directed mutagenesis (Kramer, W., et al., supra) of appropriate M13 subclones, Figure 19. Oligonucleotides were synthesized on a Cyclone DNA synthesizer, New Brunswick Scientific Co., and purified by acrylamide gel electrophoresis. A BclI site, Figure 17, located approximately 45 bp upstream of the initiation codon ATG was used for introduction of a SalI restriction site 5′ of the antibody V region. The J region mutagenesis primer 5′-GAGACGGTGACCGAGGTTCCT-3′ was used to insert a BstEII site into the M13 subclone pING3122. The restriction fragment containing the 4D12 V_H region bounded by SalI and BstEII was then cloned into the expression vector

pING2240 (pING2240 is identical to pING2227 except it contains a different antibody V region).

The cDNA clone containing the 4D12 L chain, pC5K-4, was adapted for mammalian expression in a similar way, Figure 20. The J region mutagenesis primer 5'-CAGCTCAAGCTTGGTCCC-3' used to insert a HindIII site into the M13 subclone pING3123. A BclI site, Figure 18, located approximately 30 bp upstream of the initiation codon ATG was used to introduce a SalI restriction site into pING3125 upstream of the initiation codon ATG. The restriction fragment containing the 4D12 L chain V region bounded by SalI and HindIII in pING3130 was then cloned into the expression vector pING1712 (pING2216 is identical to pING1712 except it contains a different antibody V region).

3. Stable Transfection of Mouse Lymphoid Cells for the Production of Chimeric Antibody

The Chimer5 L chain expression plasmid, pING3132, and the H chain expression plasmid, pING3126, were each linearized by digestion with PvuI restriction endonuclease and co-transfected into Sp2/0 cells, giving mycophenolic acid resistant clones which were screened for L + H chain synthesis and G418 resistance. The MPA- and G418-resistant clone producing the most L + H chain, Sp2/0-3132 + 3126 1G4, was used for production of Chimer5 for testing.

4. Purification and Testing of Chimer5 Antibody Secreted in Tissue Culture

Sp2/0-3132 + 3126 1G4 cells were grown in culture medium DMEM (GIBCO) supplemented with 5% fetal bovine serum, HEPES buffer, glutamine, penicillin, and streptomycin (Irvine Scientific, Irvine, CA). Chimer5 was purified in a similar manner to that described for Chimer4 in Example 7. Purified Chimer5 was tested to demonstrate that it retained the antigen binding characteristics of the mouse 4D12 antibody using commercially available HIV Western strips (Dupont or

equivalent) as described for Chimer1, Chimer2, and Chimer4, above. Both the Chimer5 and mouse 4D12 antibodies recognized the same HIV gag antigen.

EXAMPLE 11

A Chimeric Mouse-Human Fab Produced in E. coli and Specific for an HIV gag Protein

Bacteria are suited for production of chimeric antibodies expressed from mammalian cDNA since entire coding sequences can be expressed from well characterized promoters. The following sections detail the secretion of Chimer5 Fab from E. coli.

1. In Vitro Mutagenesis

Site directed in vitro mutagenesis was performed as described by Kramer, et al., supra, to introduce a PstI restriction site into the 4D12 L chain cDNA sequence (Figure 18) at the junction of the leader peptide and mature coding region with the oligonucleotide primer 5'-TCATCACAACATCTGCAGTGGTTTCCCGA-3'.

A ApaI site was similarly introduced at the junction of the leader peptide and mature coding region of the 4D12 H chain with the oligonucleotide primer 5'-TGGACCTGGGCCCGAACACCTGC-3' (Figure 17).

2. Preparation of L Chain for Bacterial Expression

The Chimer5 L chain V region containing a PstI restriction site at the signal sequence processing site and a unique HindIII site in the J region of pING3128 served as the starting point for bacterial expression. The plasmid pING3128 was cut with PstI, treated with T4 polymerase, and digested with HindIII (Figure 21A). The 320 bp fragment containing the V_L region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI

(Figure 21B) along with a HindIII to XhoI restriction fragment containing the human C_κ plus 15 nucleotides of 3' genomic DNA from pING3102 (Figure 21C). The resulting plasmid that contained a pelB::Chimer5 L chain fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pING3133.

3. Preparation of Fd for Bacterial Expression

The intact Chimer5 chimeric Fd gene containing a ApaI restriction site at the signal sequence processing site and a BstEII restriction site in the J region in pING3129 served as the starting point for bacterial expression. The plasmid pING3129 was cut with ApaI, treated with T4 polymerase, and digested with BstEII (Figure 21D). The approximately 335 bp fragment containing the Fd V region was purified and ligated to pING1500 that was cut with SstI, treated with T4 polymerase, and cut with XhoI (Figure 21E) along with the human CH1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D (Figure 21F). The resulting plasmid that contained a pelB::Chimer5 Fd fusion was sequenced to determine that the proper in-frame fusion was formed. This plasmid was called pING3131.

4. Multicistronic Expression System for L Chain and Fd Gene

For production of bacterially derived Fab, both L chain and Fd need to be produced simultaneously within the cell. Using the plasmids constructed with each of these genes separately, a series of expression vectors were constructed that contain both genes aligned so that transcription from a single promoter will specify both genes. This was done in a way that minimized the noncoding DNA between the two genes. Each gene has a ribosome binding site needed for translation initiation and the identical DNA sequence from -48 to the pelB leader::antibody gene junction.

Plasmid pING3133 was cut with SphI, treated with T4 polymerase, cut with EcoRI, and the vector fragment was purified (Figure 21G). Similarly, pING3131 was cut with XhoI, treated with T4 polymerase, cut with EcoRI and the fragment containing the Fd gene was purified (Figure 21H). These two purified DNA fragments were ligated to produce pING3136, which contained the two Chimer5 gene fusions linked in close proximity. The two gene cistron was placed under the control of the araB promoter in pING3107. Plasmid pING3136 was cut with SphI, treated with T4 polymerase, cut with XhoI, and the fragment containing the Fd and κ genes was purified (Figure 21I). This DNA fragment was ligated to the vector fragment from pING3107 that had been cut with EcoRI, treated with T4 polymerase, and cut with XhoI (Figure 21J), generating pING3139. This vector contains all the necessary features for expression of Chimer5 Fab in E. coli.

5. Production of Chimer5 Fab in Bacteria

Expression of Chimer5 Fab from pING3139 in E. coli is under the inducible control of the araB promoter. Upon arabinose induction, Fab secreted into the growth medium increased more than 10-fold. The E. coli strain harboring pING3139 was was cultured at 32°C in 10L of minimal medium, supplemented with 1.7% glycerol as the carbon source. Dissolved oxygen was maintained at 20% of saturation by adition of a 5X concentrate of glycerol-supplemented minimal medium. The culture was induced at OD_{600}=50 by addition of a 5X concentrate of glycerol-supplemented minimal medium containing 5g arabinose in 3L of concentrate. The induction solution was added over a period of about 20 hours, then 5X concentrate without minimal medium was added for about 4 hours. Fab was detected in the fermentation broth by ELISA.

About 7 liters of a culture supernatant was concentrated, analyzed, and stored as in Example 2.

The Chimer5 Fab purified from E. coli had identical molecular weight properties as other Fab molecules produced from E. coli, as assessed by SDS gel electrophoresis. The bacterially-produced Chimer5 Fab was correctly assembled as a κ plus Fd chain dimer because of its positive reaction in the enzyme immunoassays detecting molecules with both κ and Fd determinants, and because it reacted with core protein on commercially available test strips.

6. Study Performed on Chimer5 Fab

Chimer5 Fab was tested along with 4D12 mouse antibody and Chimer5 antibody for binding to antigen fixed to commercially available HIV Western test strips (Dupont or equivalent). Chimer5 Fab was incubated with the test strip, followed by interaction with goat anti-human Fab antibody, and then with rabbit anti-goat IgG. Detection of bound antibody was with Protein A-gold (BioRad) followed by silver enhancement. Chimer5 Fab specifically recognized the same size antigens of 55, 45, and 39 kD on the test strip as did Chimer5 whole antibody.

EXAMPLE 12

A Chimeric Mouse-Human Fab Fragment Produced in Yeast and Specific for an HIV gag Protein

Yeast cells are capable of expressing and secreting foreign proteins. In this example, yeast serve as a host for the production of mouse-human chimeric Fab. Yeast strains and growth conditions are as in Example 3.

1. Construction of Yeast Expression Plasmids Containing Antibody Genes

The gene sequences encoding the mature form of the V_L region of 4D12 and containing a HindIII site in the J region (as described in Example 11) and a PstI site introduced at the signal sequence processing site was fused to the human C_κ

region. Plasmid pING3128 was cut with PstI, treated with T4 polymerase, and cut with HindIII. The V region fragment was ligated along with the human C_L region prepared from pING3102 as a HindIII to XhoI fragment into pING1149. The plasmid pING1149 contains the gene sequence encoding the yeast invertase signal sequence (Taussig, R. et al., supra) under control of the yeast PGK promoter, Hitzeman, R.A., et al., supra). The resultant plasmid pING3134, (similar to Figure 6A), contained the PGK promoter (P), along with the invertase signal sequence (S), fused to the Chimer5 coding sequence. As the result of this fusion, the gene sequence encoding the mature form of the Chimer5 L chain was fused in frame to the gene sequence encoding the yeast invertase signal sequence. The PGK promoter - invertase signal sequence - chimeric L chain (V,C_κ) fusion was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK polyadenylation signal (Tm) to generate pING3140 (similar to Figure 6C). The gene sequence encoding the mature form of the V_H region of Chimer5 containing a BstEII site in the J region (as described in Example 11) and a ApaI site introduced at the signal sequence processing site from pING3129 was fused along with the human C_H1 region (which had been previously generated by introducing a stop codon in hinge, Robinson, R.R., et al., supra) from pF3D into pING1149 that had been cut with PstI, treated with T4 polymerase and cut with XhoI. This generated plasmid pING3135. The PGK promoter - invertase signal sequence - chimeric Fd chain (V,C_H1) fusion (similar to Figure 6B) was cloned into a partial 2 micron circle (2μ), ura3 yeast expression vector containing the PGK polyadenylation signal (Tm) to generate pING3141 (similar to Figure D).

A single yeast expression vector containing both the chimeric L chain and Fd chain genes and their respective expression signals was constructed from pING3140 and pING3141. This final vector, pING3143, similar to Figure 6E, contains a

portion of 2 micron circle (oriY, REP3) and the two selectable markers leu2d and ura3.

2. Yeast Secretion of Chimer5 Fab

The plasmid pING3143 is transformed into S. cerevisiae PS6 and the transformants are grown in broth under selective conditions as described above. Transformed yeast cultures secrete Chimer4 Fab into the culture medium, and this material is purified from fermentation broth and is expected to have properties identical to those of Fab produced as described in Example 3; yeast Chimer5 Fab is expected to have the same binding specificity as Fab produced in E. coli.

CONCLUSIONS

The examples presented above demonstrate a number of important qualities of the Chimer1, Chimer2, Chimer4, and Chimer5 antibodies and the genetically engineered fragments and derivatives of the invention. First, the chimeric antibodies, fragments and derivatives bind to the same antigens as the parent mouse mAbs. This is evidenced by direct binding of chimeric antibodies, fragments and derivatives to Western immunoblot test strips prepared with lysates of HIV.

To date there has been only very limited testing of antibody therapy for the treatment of HIV-infected individuals. Passive immunization of these patients with pooled high-titer human serum has resulted in some clinical improvement. Direct therapy with mouse mAbs is not likely to be feasible since mouse antibodies are known to elicit a strong immune response against themselves. The chimeric antibodies of this invention represent potentially important agents for treatment of HIV infection. Antibodies such as Chimer1, Chimer2, Chimer 4, and Chimer 5 or their corresponding fragments and derivatives, either alone, as

immunoconjugates, or in combination with other agents may be advantageously used for HIV treatment.

The chimeric antibodies of this invention may also be used for any diagnostic purpose for which the similar mouse antibodies or their derivatives can be used. Furthermore, the chimeric antibodies, fragments and derivatives may be used as immobilized reagents for ex vivo adsorption of viruses, viral antigens, or virus-infected cells.

Chimeric antibody molecules, such as those of the present invention, and their derivatives, embody a combination of the advantageous characteristics of mouse and human mAbs. Like mouse mAbs, they can recognize and bind to viral antigens; however, unlike mouse mAbs, the "human specific" properties of chimeric antibodies lower the likelihood of an immune response to the antibodies, and result in prolonged survival in the circulation through reduced clearance. These properties have been observed when a chimeric antibody directed gainst a tumor marker was introduced in patients, LoBuglio, et al., 1989, Proc. Natl. Acad. Sci. USA 86:4220-4224.

The human component of a chimeric antibody may enhance its ability to mediate target destruction, for example, of virally infected cells, in combination with human effector cells or complement. Moreover, using the methods disclosed in the present invention, any desired antibody isotype can be combined with any particular antigen combining site. This invention also enables the direct production of one or more domains of the antibody molecule in functionally active form.

DEPOSITS

Illustrative cell lines secreting Chimer1, Chimer2, Chimer4, and Chimer5 antibodies were deposited on October 25, 1989 at the ATCC, Rockville Maryland.

The E. coli strains deposited are as follows:

1. E. coli MC1061 transformed with pING3127 (G269) was granted ATCC Accession #68146.
2. E. coli MC1061 transformed with pING3211 (G270) was granted ATCC Accession #68147.
3. E. coli MC1061 transformed with pING3405 (G271) was granted ATCC Accession #68148.
4. E. coli MC1061 transformed with pING3139 (G272) was granted ATCC Accession #68149.

The transfected hypbridoma cell lines are as follows:

1. $S_p/0$ (pING3110 + pING3112) (C863) was granted ATCC Accession # HB10277.
2. $S_p/0$ (pING3005 + pING3004) (C850) was granted ATCC Accession # HB10276.
3. $S_p/0$ (pING2258 + pING2255) (C871) was granted ATCC Accession # HB10279.
4. $S_p/0$ (pING3132 + pING3126) (C864) was granted ATCC Accession # HB10278.

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 5, line 4 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☒ [3]

Name of depositary institution [4]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852
~~United States of America~~

Date of deposit [5]	Accession Number [6]
25 October 1989	ATCC 68146

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Escherichia coli MC1061 pING3127, G269

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

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MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 6, line 7 of the description

A. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country)

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
25 October 1989	ATCC 68147

B. ADDITIONAL INDICATIONS (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Escherichia coli MC1061 pING3211, G270

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications e.g., "Accession Number of Deposit")

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MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 42, line 17 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet [X] [3]

Name of depositary institution [4]

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Address of depositary institution (including postal code and country) [4]

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United States of America

Date of deposit [5]	Accession Number [6]
25 October 1989	ATCC 68148

B. ADDITIONAL INDICATIONS [1] (leave blank if not applicable). This information is continued on a separate attached sheet [ ]

Escherichia coli MC1061 pING3405, G271

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [1] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [1] (leave blank if not applicable)

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MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 7, line 25 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet [3] ☒

Name of depositary institution [4]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [4]

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Rockville, Maryland 20852
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Date of deposit [5]	Accession Number [6]
25 October 1989	ATCC 68149

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Escherichia coli MC1061 pING3139, G272

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

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MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 32, line 9 of the description [5]

A. IDENTIFICATION OF DEPOSIT [6]

Further deposits are identified on an additional sheet [6] ☐

Name of depositary institution [6]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [6]

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit [6]	Accession Number [6]
25 October 1989	ATCC HB 10276

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Mouse Sp2/0 Hybridoma Cells Carrying Plasmids pING3005 and pING3004, C850

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

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MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 20 line 26 of the description [5]

A. IDENTIFICATION OF DEPOSIT [6]

Further deposits are identified on an additional sheet [6] ☐

Name of depositary institution [4]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852
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Date of deposit [6]	Accession Number [6]
25 October 1989	ATCC HB 10277

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Mouse Sp2/0 Hybridoma Cells Carrying Plasmids pING3110 and pING3112, C863

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

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International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 46, line 28 of the description [1]

A. IDENTIFICATION OF DEPOSIT [6]

Further deposits are identified on an additional sheet [X] [6]

Name of depositary institution [4]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [4]

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Rockville, Maryland 20852
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Date of deposit [5]	Accession Number [6]
25 October 1989	ATCC HB 10278

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet [ ]

Mouse Sp2/0 Hybridoma Cells Carrying Plasmids pING3132 and pING3126, C864

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

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International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 40, line 8 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet [2] ☒

Name of depositary institution [4]

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution (including postal code and country) [5]

12301 Parklawn Drive
Rockville, Maryland 20852
~~United States of America~~

Date of deposit [6]	Accession Number [7]
25 October 1989	ATCC HB 10279

B. ADDITIONAL INDICATIONS [3] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

Mouse Sp2/0 Hybridoma Cells Carrying Plasmids pING2258 and pING2255, C871

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

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WHAT IS CLAIMED IS:

1. A polynucleotide molecule comprising a nucleotide sequence coding for at least part of the variable region of an immunoglobulin chain derived from an antibody selected from the group consisting of the monoclonal antibodies designated 2E12, 2G12, 1C11, and 4D12.

2. The molecule of claim 1 which is a recombinant DNA molecule.

3. The molecule of claim 2 in double stranded DNA form.

4. The molecule of claim 3 which is an expression vehicle.

5. The molecule of claim 4 wherein said vehicle is a plasmid.

6. A prokaryotic host transformed with the molecule of claim 1.

7. The host of claim 6 which is a bacterium.

8. A eukaryotic host transfected with the molecule of claim 1.

9. The host of claim 8 which is a yeast cell or a mammalian cell.

10. The molecule of claim 1 wherein said immunoglobulin chain is a heavy chain.

11. The molecule of claim 1 wherein said immunoglobulin chain is a light chain.

```
                    SalI
3'-GCGGCCTGAGACAGCTGGTTCAAGAGT-5'

                                        METArgCysSerLeuGlnPheLeuGly
GGGGCGCCGGACTCTTCACACCAAGTTCTCAGAATGAGGTGCTCTCTTCAGTTCCTGGGG
          15                  30                  45                  60

                        BsmI
              3'-TAGAGACCTCACTTACGCCTCTAACACT-5'
ValLeuMETPheTrpIleSerGlyValSerGlyGluIleValIleThrGlnAspGluLeu
GTGCTTATGTTCTGGATCTCTGGAGTCAGTGGGGAGATTGTGATAACCCAGGATGAACTC
          75                  90                  105                 120

SerAsnProValThrSerGlyGluSerValSerPheSerCysArgSerSerLysSerLeu
TCCAATCCTGTCACTTCTGGAGAATCAGTTTCCTTCTCCTGCAGGTCTAGTAAGAGTCTC
          135                 150                 175                 180

LeuTyrLysAspGlyLysThrTyrLeuSerTrpPheLeuGlnArgProGlyGlnSerPro
CTATATAAGGATGGGAAGACATACTTGAGTTGGTTTCTGCAGAGACCAGGACAATCTCCG
          195                 210                 225                 240
```

FIG. 1

GlnLeuLeuIleTyrLeuMETSerThrArgValSerGlyValSerAspArgPheSerGly
CAGCTCCTGATCTATCTGATGTCCACCCGTGTATCAGGAGTCTCAGACCGGTTTAGTGGC
255 270 285 300

SerGlySerGlyThrAspPheThrLeuGluIleSerGlyValLysAlaGluAspValGly
AGTGGGTCAGGAACAGATTTCACCCTGGAAATCAGTGGAGTGAAGGCTGAGGATGTGGGT
315 330 345 360

HindIII
3'-CCTGGTTCGAA
ValTyrTyrCysGlnGlnLeuValGluTyrProTyrThrPheGlyGlyGlyThrLysLeu
GTGTATTACTGTCAACAACTTGTAGAGTATCCGTACACATTCGGAGGGGGGACCAAGCTG
375 390 405 420

CTTTATTTTG-5'
GluIleLys
GAAATAAAA

FIG. 1(cont.)

SalI

3′ TGTCAATGATTCAGCTGTCCTGGAG-5′ METGlyTrpSerCys

AAATCACTGTTCTCTCTACAGTTACTAAGTACACAGGACCTCACCATGGGATGGAGCTGT

15 30 45 60

KpnI

3′-GATGTCCACAGGCCATGGTCCACGTTG-5′

IleIleLeuPheLeuValSerThrAlaThrGlyValHisSerGlnValGlnLeuGlnGln

ATCATCCTCTTCTTGGTATCAACAGCTACAGGTGTCCACTCCCAGGTGCAACTGCAGCAG

75 90 105 120

SerGlyProGlnLeuValArgProGlyAlaSerValLysIleSerCysLysAlaSerGly

TCTGGGCCTCAGCTGGTTAGGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGT

135 150 165 180

TyrSerPheThrAsnTyrTrpIleHisTrpValAsnGlnArgProGlyGlnGlyLeuGlu

TACTCATTCACCAACTACTGGATACACTGGGTGAATCAGAGGCCTGGACAAGGTCTTGAG

195 210 225 240

FIG. 2

```
TrpIleGlyMETIleAspProSerAspSerGluThrArgLeuThrGlnLysPheLysAsp
TGGATTGGCATGATTGATCCTTCCGATAGTGAAACTAGGTTAACTCAGAAGTTCAAGGAC
          255                 270                 285                 300

LysValThrLeuThrValAspLysSerSerAsnThrAlaTyrLeuGlnLeuSerSerPro
AAGGTCACATTGACTGTAGACAAATCCTCCAACACAGCCTACCTGCAACTCAGCAGCCCG
          315                 330                 345                 360

ThrSerGluAspSerAlaValTyrTyrCysAlaArgSerAspTyrGlyPheAspSerTrp
ACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCAGACTATGGTTTTGACTCCTGG
          375                 390                 405                 420

                BstEII
      3'-GGTGCCAGTGGCAGAGGAGTCGG-5'
GlyGlnGlyThrThrLeuThrValSerSerAlaLysThrThrProProSerValTyr
GGCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTAT
          435                 450                 465
```

FIG. 2 (cont.)

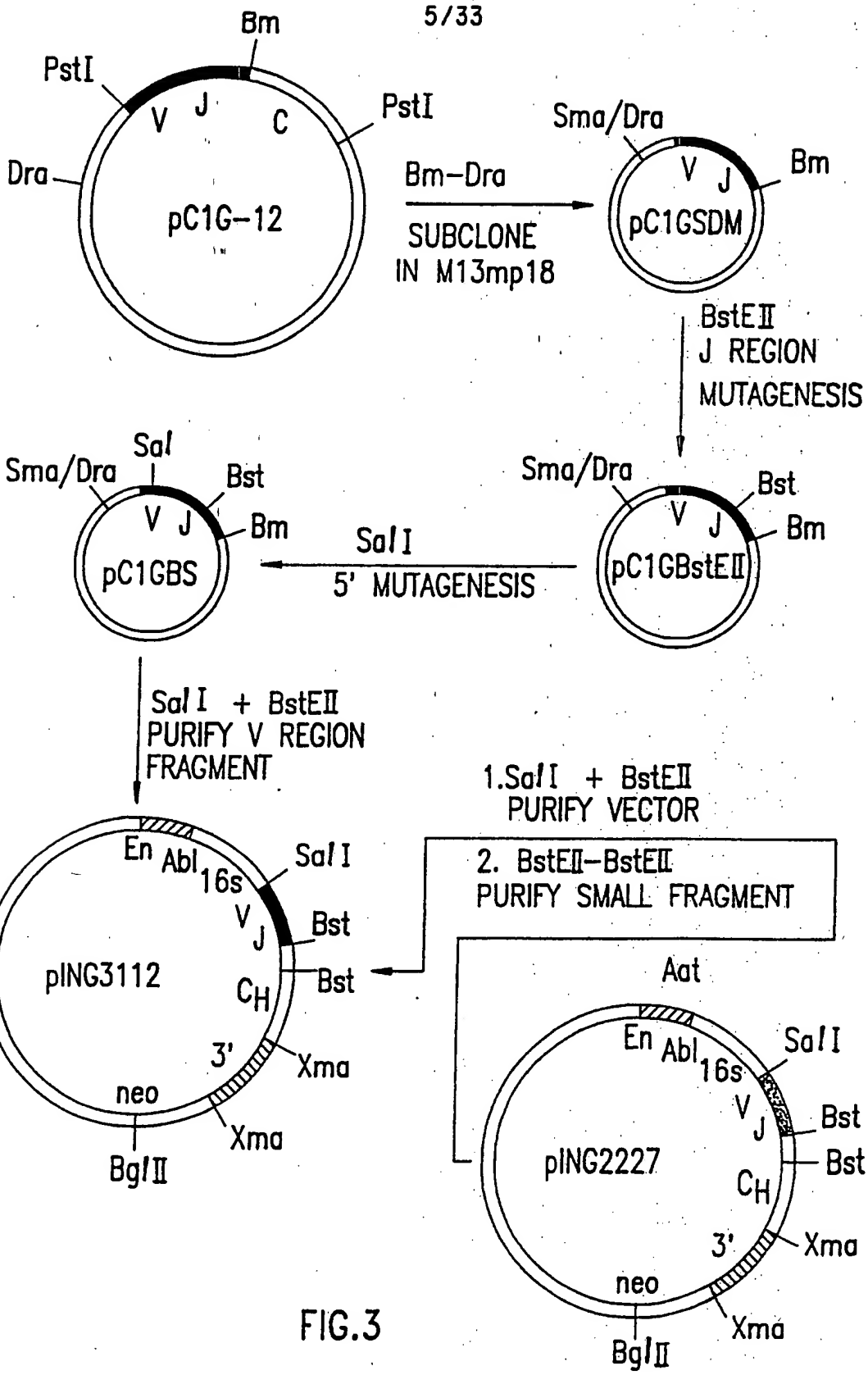
Bm
PstI
V J C
PstI
Dra
pC1G-12
Bm-Dra
SUBCLONE
IN M13mp18
Sma/Dra
V J
Bm
pC1GSDM
BstEII
J REGION
MUTAGENESIS
Sal
Sma/Dra
Bst
V J
Bm
pC1GBS
SalI
5' MUTAGENESIS
Sma/Dra
Bst
V J
Bm
pC1GBstEII
SalI + BstEII
PURIFY V REGION
FRAGMENT
1.SalI + BstEII
PURIFY VECTOR
2. BstEII-BstEII
PURIFY SMALL FRAGMENT
En Abl 16s
SalI
V J
Bst
pING3112
CH
Bst
3'
Xma
neo
Xma
BglII
Aat
En Abl 16s
SalI
V J
Bst
pING2227
CH
Bst
3'
Xma
neo
Xma
BglII

FIG.3

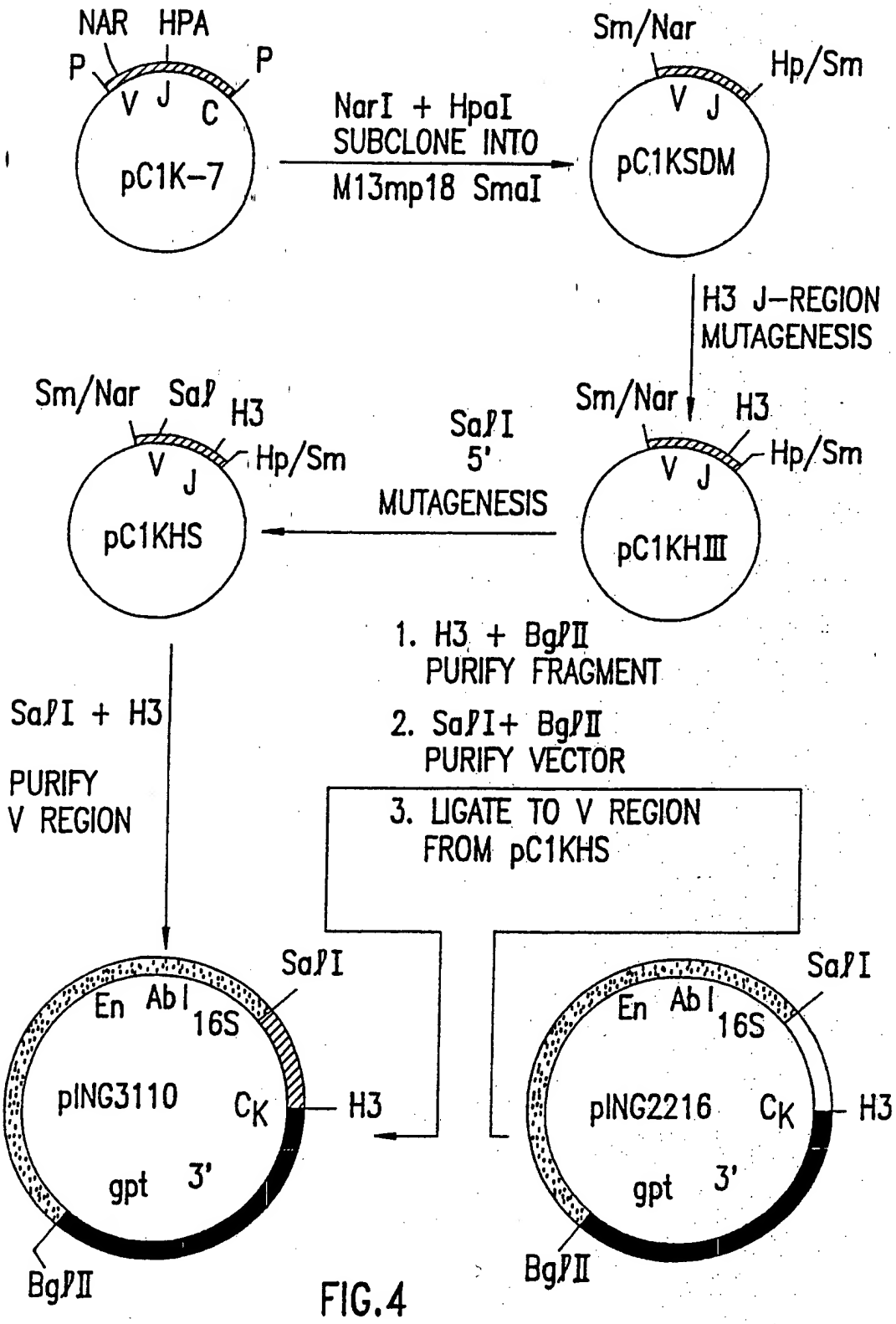
NAR HPA
P
P
V J C
pC1K-7
NarI + HpaI
SUBCLONE INTO
M13mp18 SmaI
Sm/Nar
Hp/Sm
V J
pC1KSDM
H3 J-REGION
MUTAGENESIS
Sm/Nar SalI H3
Hp/Sm
V J
pC1KHS
SalI
5'
MUTAGENESIS
Sm/Nar
H3
Hp/Sm
V J
pC1KHIII
1. H3 + BglII
PURIFY FRAGMENT
2. SalI+ BglII
PURIFY VECTOR
3. LIGATE TO V REGION
FROM pC1KHS
SalI + H3
PURIFY
V REGION
SalI
En Abl
16S
pING3110
CK
H3
gpt 3'
BglII
SalI
En Abl
16S
pING2216
CK
H3
gpt 3'
BglII

FIG.4

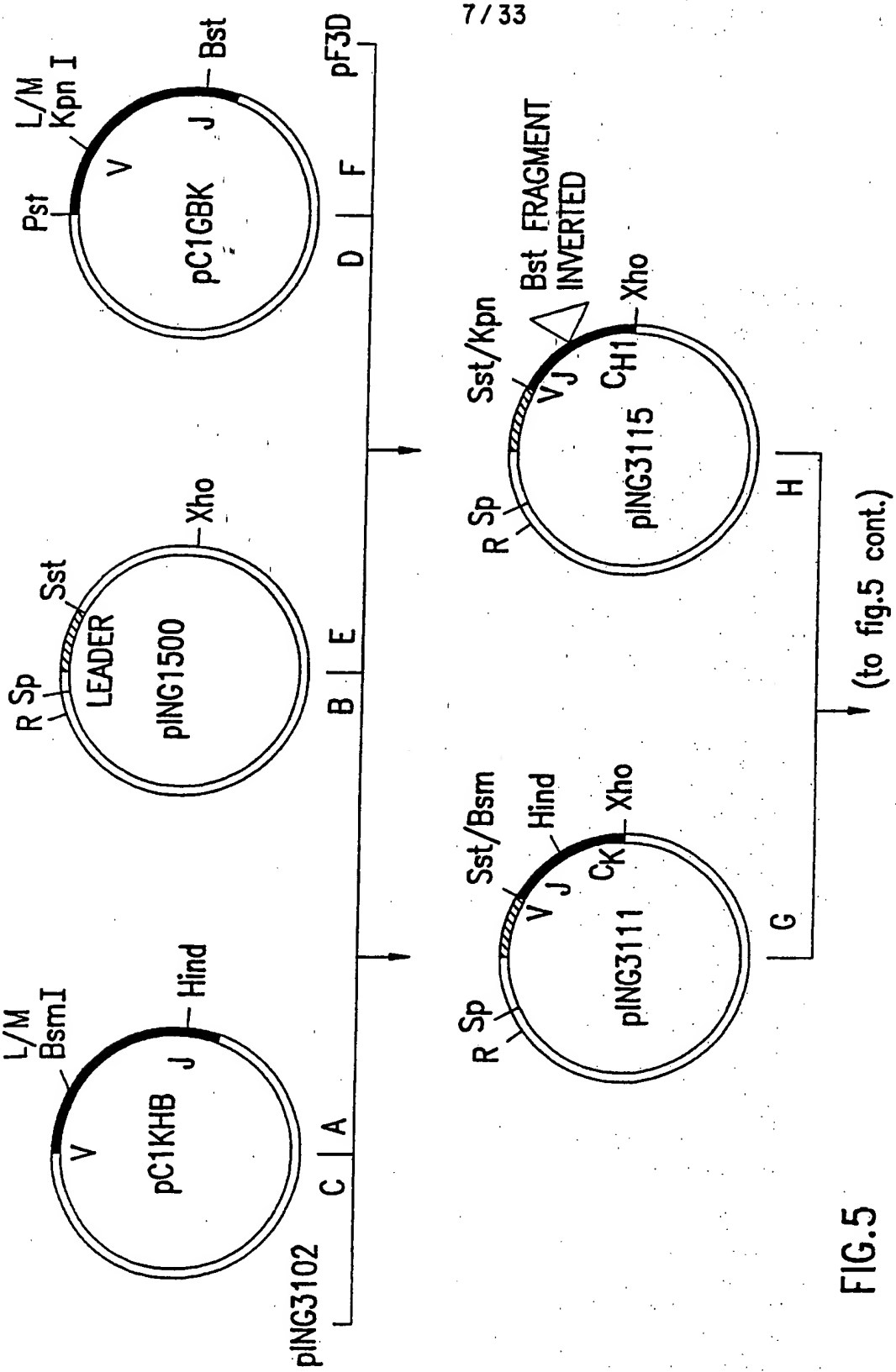
pC1GBK
Pst
L/M
Kpn I
V
J
Bst
pF3D
D
F
pC1KHB
L/M
BsmI
V
J
Hind
pING3102
C
A
pING1500
R Sp
LEADER
Sst
Xho
B
E
pING3115
R Sp
Sst/Kpn
VJ
CH1
Bst FRAGMENT
INVERTED
Xho
H
pING3111
R Sp
Sst/Bsm
V J
CK
Hind
Xho
G
(to fig.5 cont.)

FIG.5

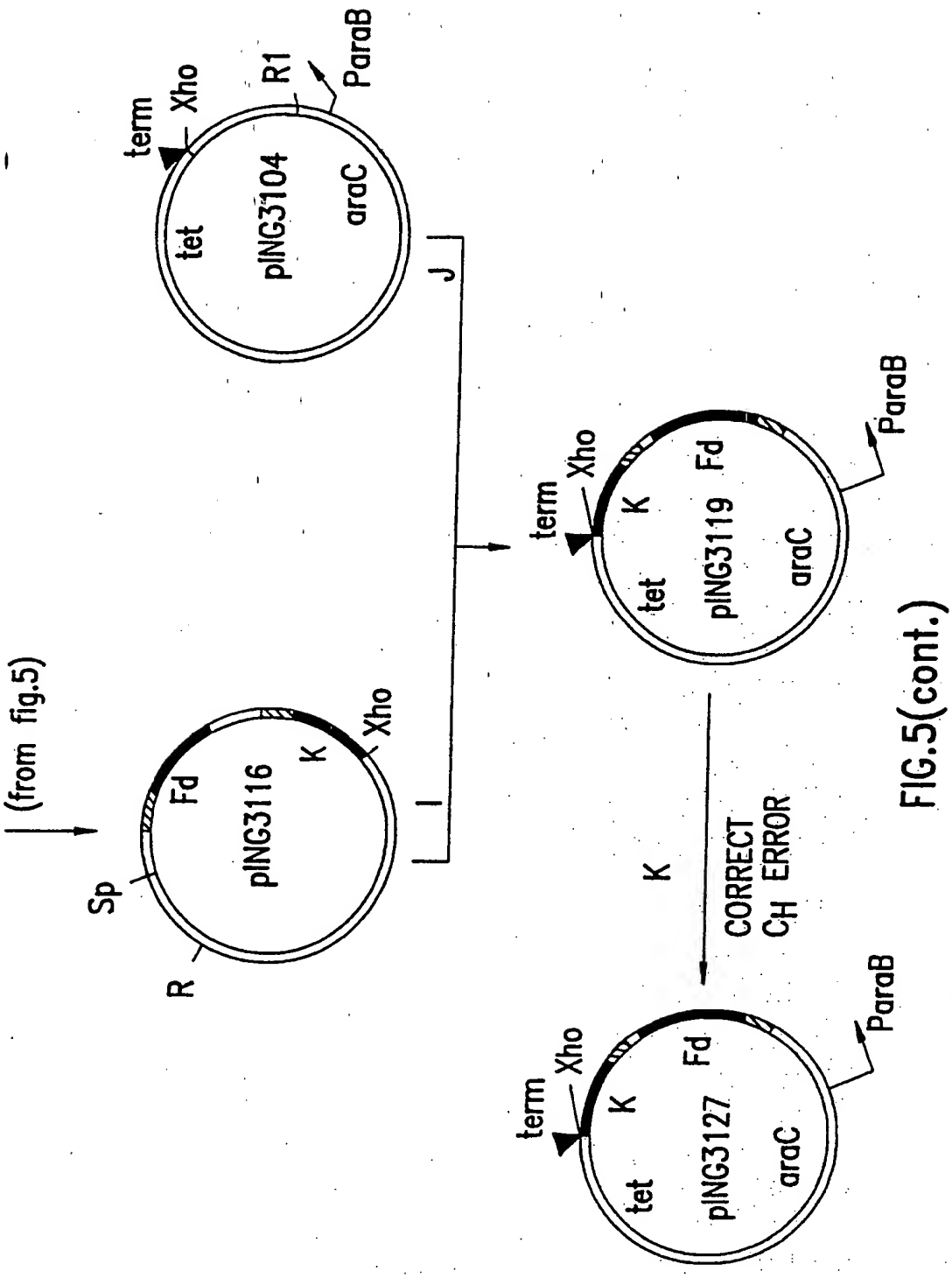
(from fig.5)
Sp
R
pING3116
Fd
K
Xho
I
J
term
Xho
tet
pING3104
araC
R1
ParaB
K
CORRECT
C_H ERROR
term
Xho
K
Fd
tet
pING3119
araC
ParaB
term
Xho
K
Fd
tet
pING3127
araC
ParaB

FIG.5(cont.)

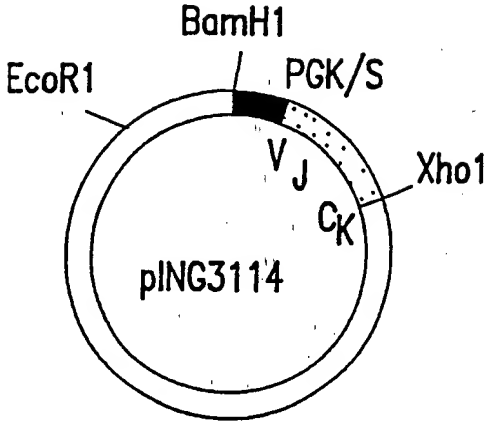

FIG.6a

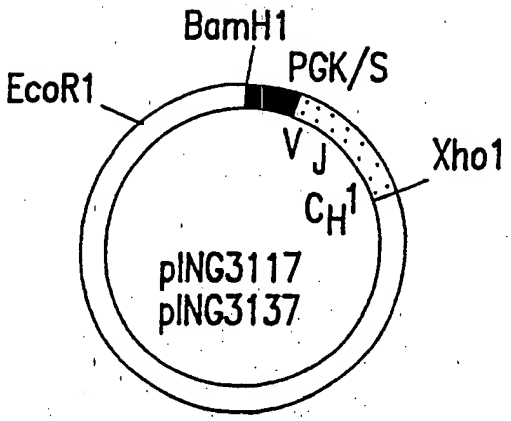

FIG.6b

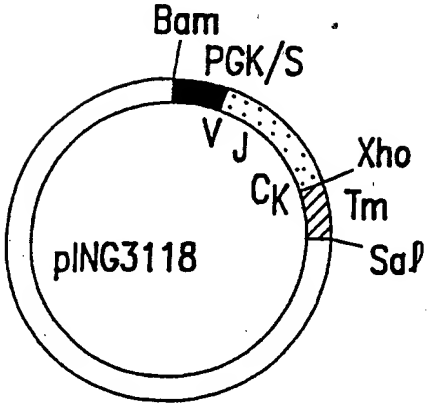

FIG.6c

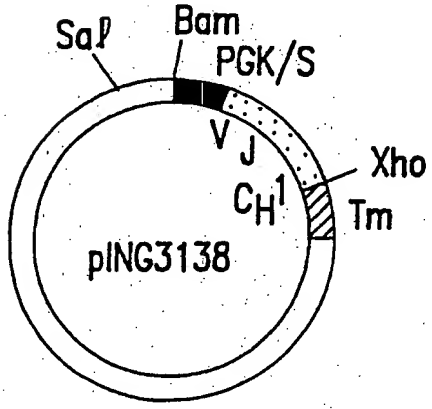

FIG.6d

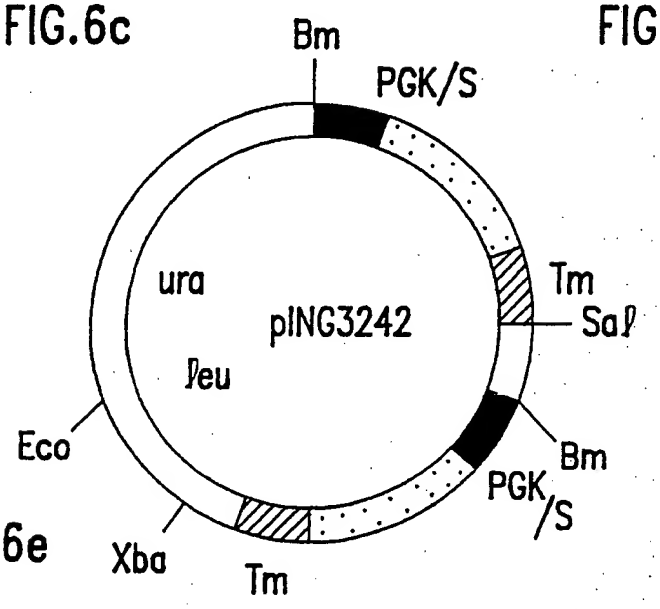

FIG.6e

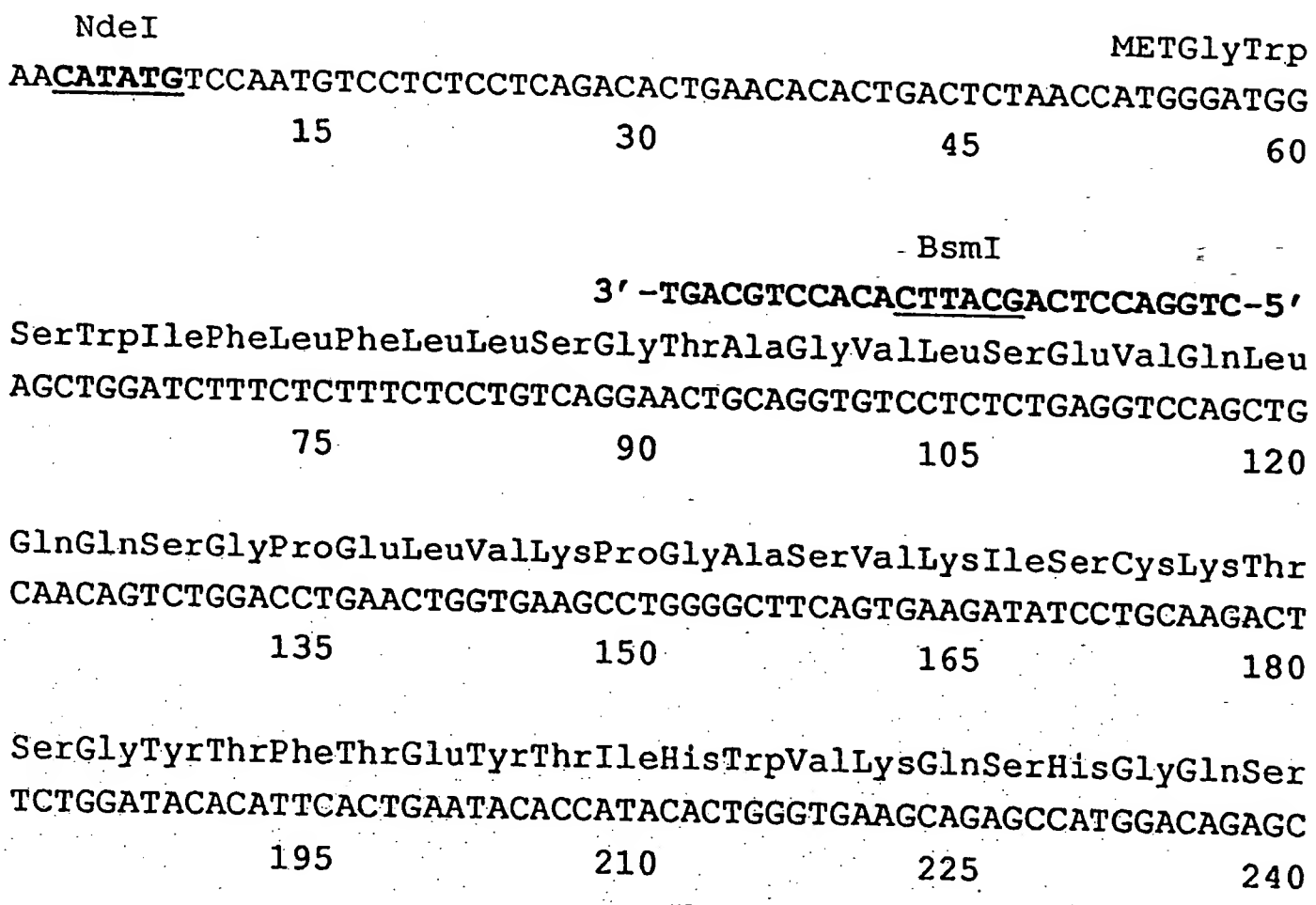
NdeI
METGlyTrp
AACATATGTCCAATGTCCTCTCCTCAGACACTGAACACACTGACTCTAACCATGGGATGG
15
30
45
60
BsmI
3'-TGACGTCCACACTTACGACTCCAGGTC-5'
SerTrpIlePheLeuPheLeuLeuSerGlyThrAlaGlyValLeuSerGluValGlnLeu
AGCTGGATCTTTCTCTTTCTCCTGTCAGGAACTGCAGGTGTCCTCTCTGAGGTCCAGCTG
75
90
105
120
GlnGlnSerGlyProGluLeuValLysProGlyAlaSerValLysIleSerCysLysThr
CAACAGTCTGGACCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGACT
135
150
165
180
SerGlyTyrThrPheThrGluTyrThrIleHisTrpValLysGlnSerHisGlyGlnSer
TCTGGATACACATTCACTGAATACACCATACACTGGGTGAAGCAGAGCCATGGACAGAGC
195
210
225
240

FIG. 7

```
LeuGluTrpIleGlyGlyIleAsnProAsnAsnGlyGlyThrThrTyrAsnGlnLysPhe
CTTGAGTGGATTGGAGGTATTAATCCTAACAATGGTGGTACTACCTACAACCAGAAGTTC
        255       270       285       300

LysAspLysAlaThrLeuIleValAspLysSerSerSerThrAlaTyrMETAspValArg
AAGGACAAGGCCACATTGATTGTAGACAAGTCCTCCAGCACAGCCTACATGGACGTCCGC
        315       330       345       360

SerLeuThrSerAspAspSerAlaValTyrTyrCysAlaArgArgGlyAsnLeuTyrTyr
AGCCTGACATCTGATGATTCTGCAGTCTATTACTGTGCAAGAGAGGAAATCTCTACTAT
        375       390       405       420

                    BstEII
3'-TCCCTGAGACCAGTGGCAGAG-5'
GlyAsnPheTrpPheAlaTyrTrpGlyGlnGlyProLeuValThrValSerAla
GGTAACTTTTGGTTTGCTTACTGGGGCCAAGGGCCTCTGGTCACTGTCTCTGCA
        435       450       465
```

FIG. 7 (cont.)

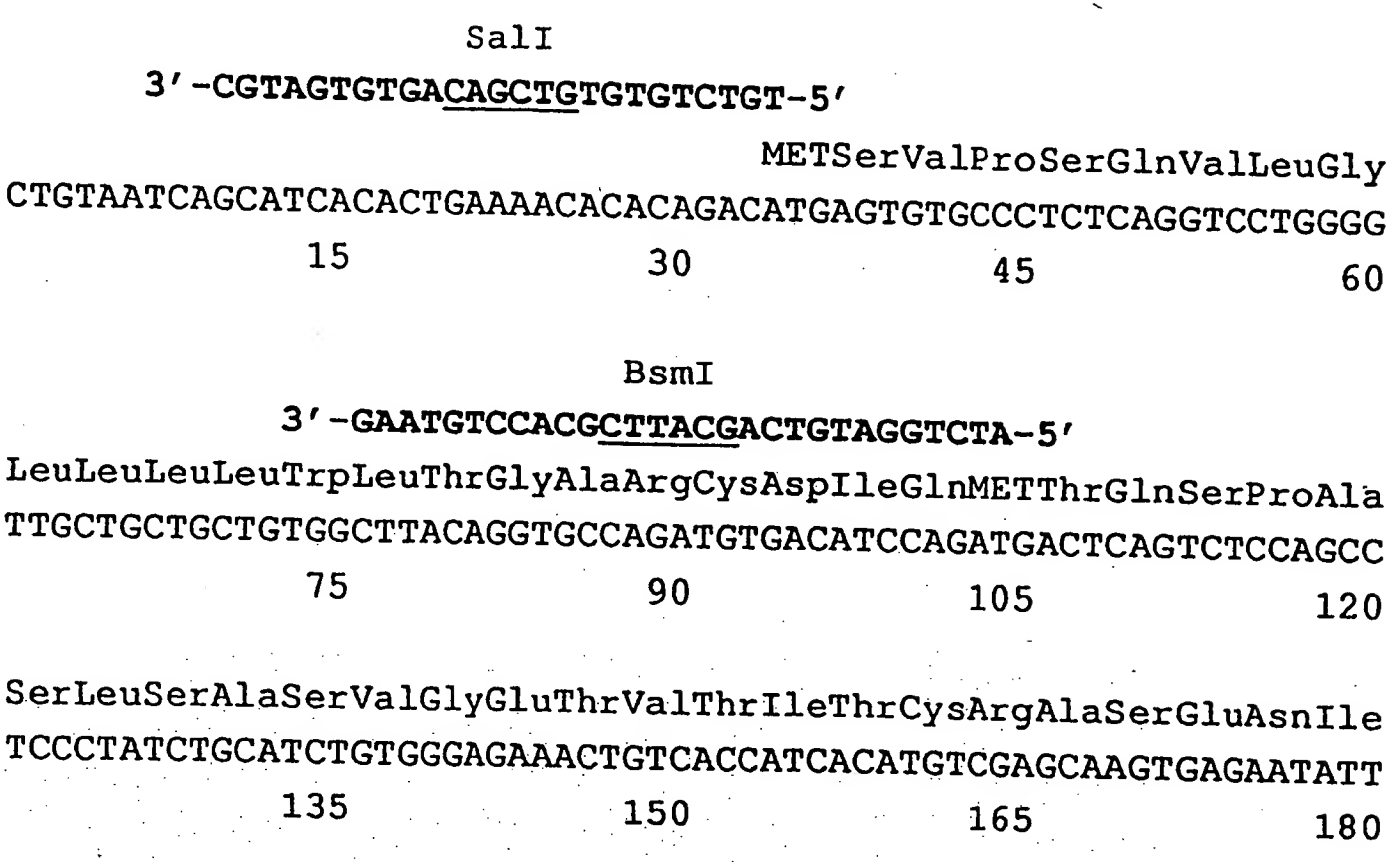
SalI
3′-CGTAGTGTGACAGCTGTGTGTCTGT-5′
METSerValProSerGlnValLeuGly
CTGTAATCAGCATCACACTGAAAACACACAGACATGAGTGTGCCCTCTCAGGTCCTGGGG
15
30
45
60
BsmI
3′-GAATGTCCACGCTTACGACTGTAGGTCTA-5′
LeuLeuLeuLeuTrpLeuThrGlyAlaArgCysAspIleGlnMETThrGlnSerProAla
TTGCTGCTGCTGTGGCTTACAGGTGCCAGATGTGACATCCAGATGACTCAGTCTCCAGCC
75
90
105
120
SerLeuSerAlaSerValGlyGluThrValThrIleThrCysArgAlaSerGluAsnIle
TCCCTATCTGCATCTGTGGGAGAAACTGTCACCATCACATGTCGAGCAAGTGAGAATATT
135
150
165
180

FIG. 8

```
TyrSerTyrLeuGlyTrpTyrGlnGlnLysGln###LysSerProGlnLeuLeuValTyr
TACAGTTATTTAGGATGGTATCAGCAGAAACAGGNAAAATCTCCTCAGCTCCTGGTCTAT
          195                 210                 225                 240

AsnAlaLysThrLeuAlaGluGlyValProSerArgPheSerGlySerGlySerGlyThr
AATGCAAAAACCTTAGCAGAAGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCACA
          255                 270                 285                 300

GlnPheSerLeuLysIleAsnArgLeuGlnProGluAspPheGlySerTyrTyrCysGln
CAGTTTTCTCTGAAGATCAACCGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGTCAA
          315                 330                 345                 360

                                                HindIII
                                    3'-CGTGGTTCGAACTTTAGTTTG-5'
HisHisTyrGlyAlaProProThrPheGlyGlyGlyThrLysLeuGluIleGln
CATCATTATGGTGCTCCTCCGACGTTCGGTGGAGGCACCAAGCTGGAAATCCAA
          375                 390                 405
```

FIG. 8(cont.)

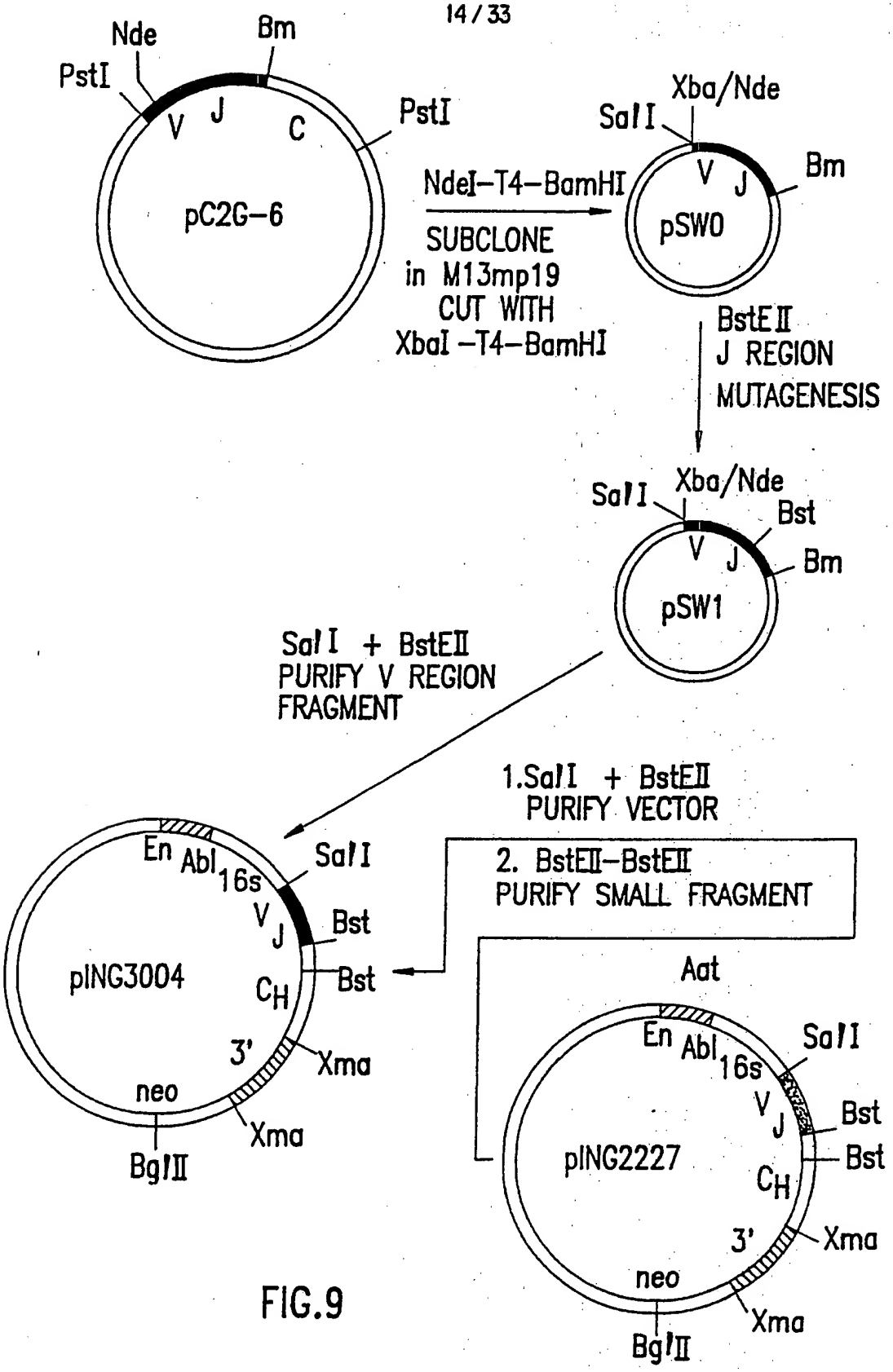
Nde
Bm
PstI
V
J
C
PstI
pC2G-6
NdeI-T4-BamHI
SUBCLONE
in M13mp19
CUT WITH
XbaI -T4-BamHI
Xba/Nde
SalI
V
J
Bm
pSW0
BstEII
J REGION
MUTAGENESIS
SalI
Xba/Nde
Bst
V
J
Bm
pSW1
SalI + BstEII
PURIFY V REGION
FRAGMENT
1.SalI + BstEII
PURIFY VECTOR
2. BstEII-BstEII
PURIFY SMALL FRAGMENT
En
Abl
16s
V
J
SalI
Bst
pING3004
CH
Bst
3'
Xma
neo
Xma
BglII
Aat
En
Abl
16s
SalI
V
J
Bst
pING2227
CH
Bst
3'
Xma
neo
Xma
BglII

FIG.9

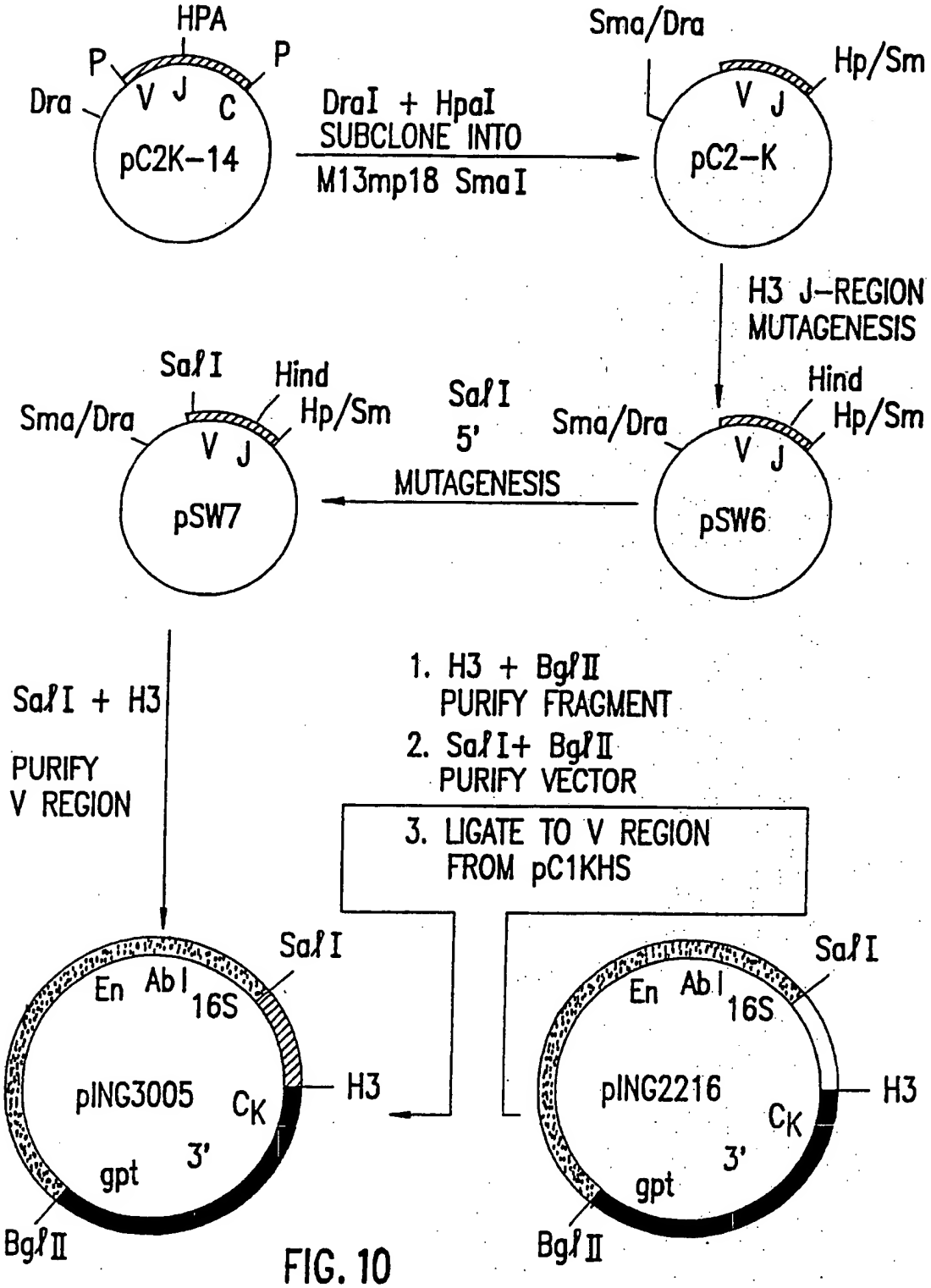
HPA
P
P
Dra
V J C
pC2K-14
DraI + HpaI
SUBCLONE INTO
M13mp18 SmaI
Sma/Dra
Hp/Sm
V J
pC2-K
H3 J-REGION
MUTAGENESIS
SalI
Hind
Sma/Dra
Hp/Sm
V J
pSW7
SalI
5'
MUTAGENESIS
Hind
Sma/Dra
Hp/Sm
V J
pSW6
SalI + H3
PURIFY
V REGION
1. H3 + BglII
PURIFY FRAGMENT
2. SalI+ BglII
PURIFY VECTOR
3. LIGATE TO V REGION
FROM pC1KHS
SalI
En
Ab I
16S
pING3005
CK
H3
3'
gpt
BglII
SalI
En
Ab I
16S
pING2216
CK
H3
3'
gpt
BglII

FIG. 10

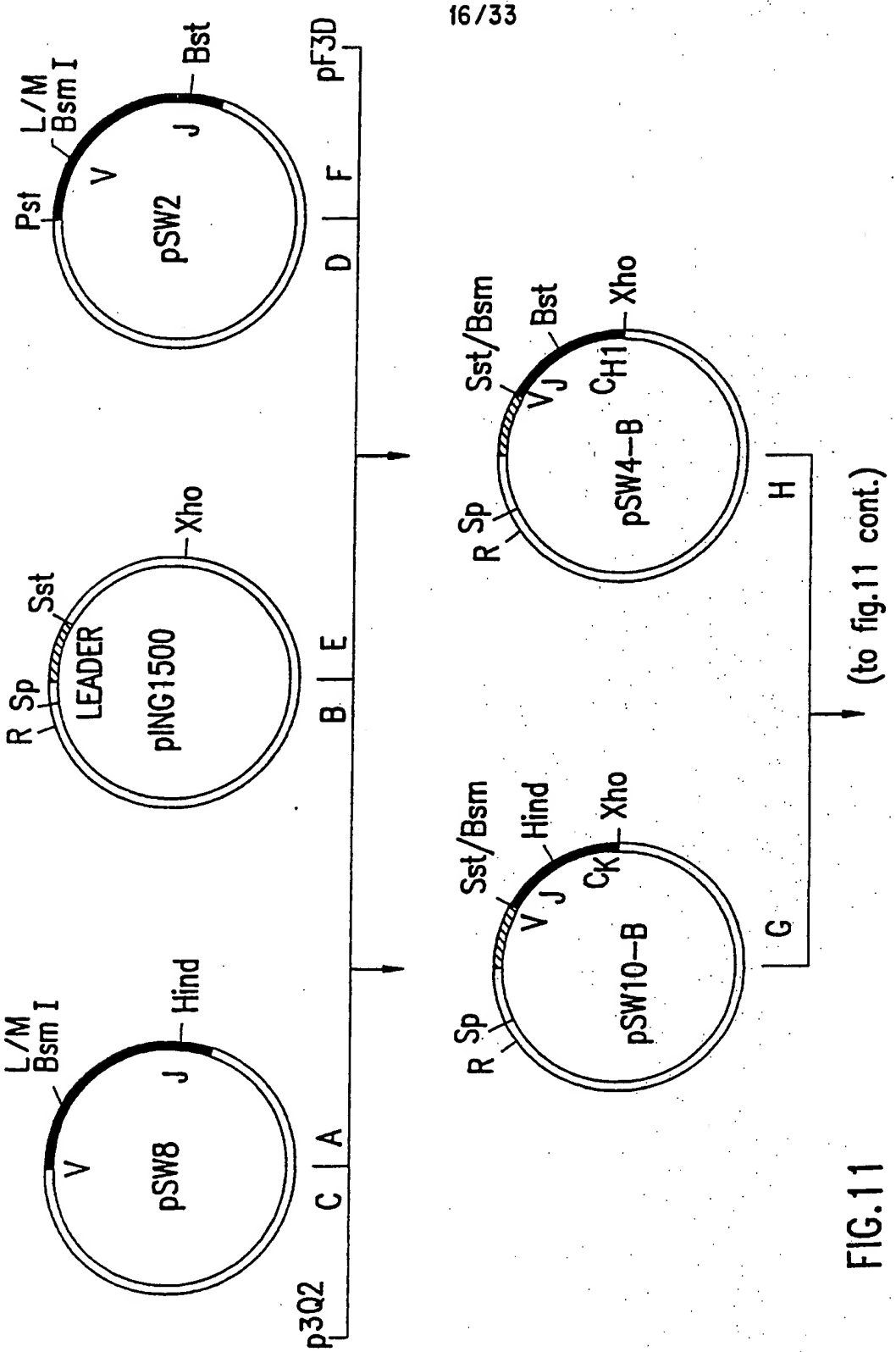
pF3D
L/M
Bsm I
Bst
Pst
V
J
pSW2
D
F
Xho
Sst
R Sp
LEADER
pING1500
B
E
Sst/Bsm
Bst
Xho
V J
CH1
R Sp
pSW4-B
H
(to fig.11 cont.)
L/M
Bsm I
Hind
V
J
pSW8
C
A
p3Q2
Sst/Bsm
Hind
Xho
V J
CK
R Sp
pSW10-B
G

FIG.11

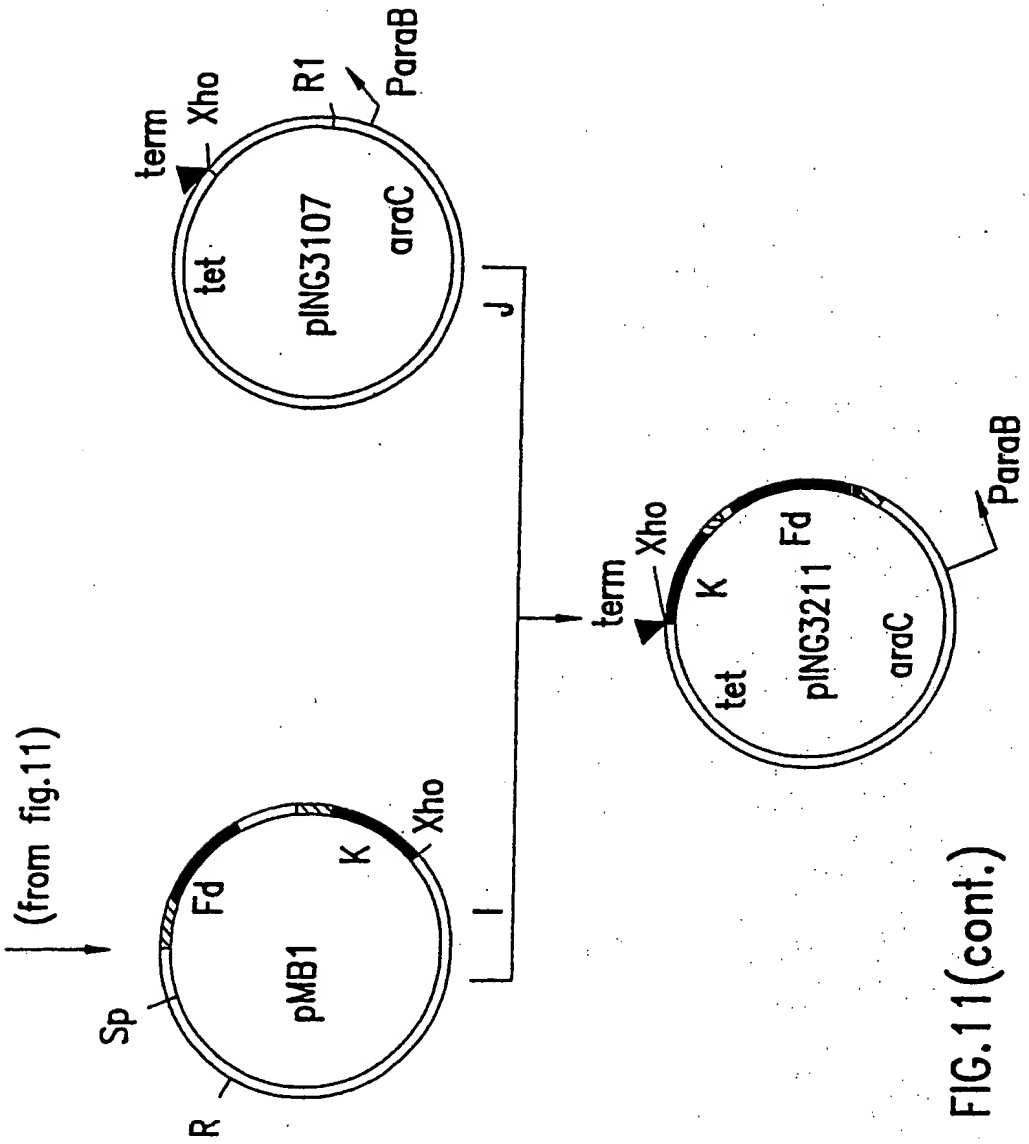
(from fig.11)
Sp
R
pMB1
Fd
K
Xho
I
J
term
Xho
R1
ParaB
tet
pING3107
araC
term
Xho
K
Fd
ParaB
tet
pING3211
araC

FIG.11(cont.)

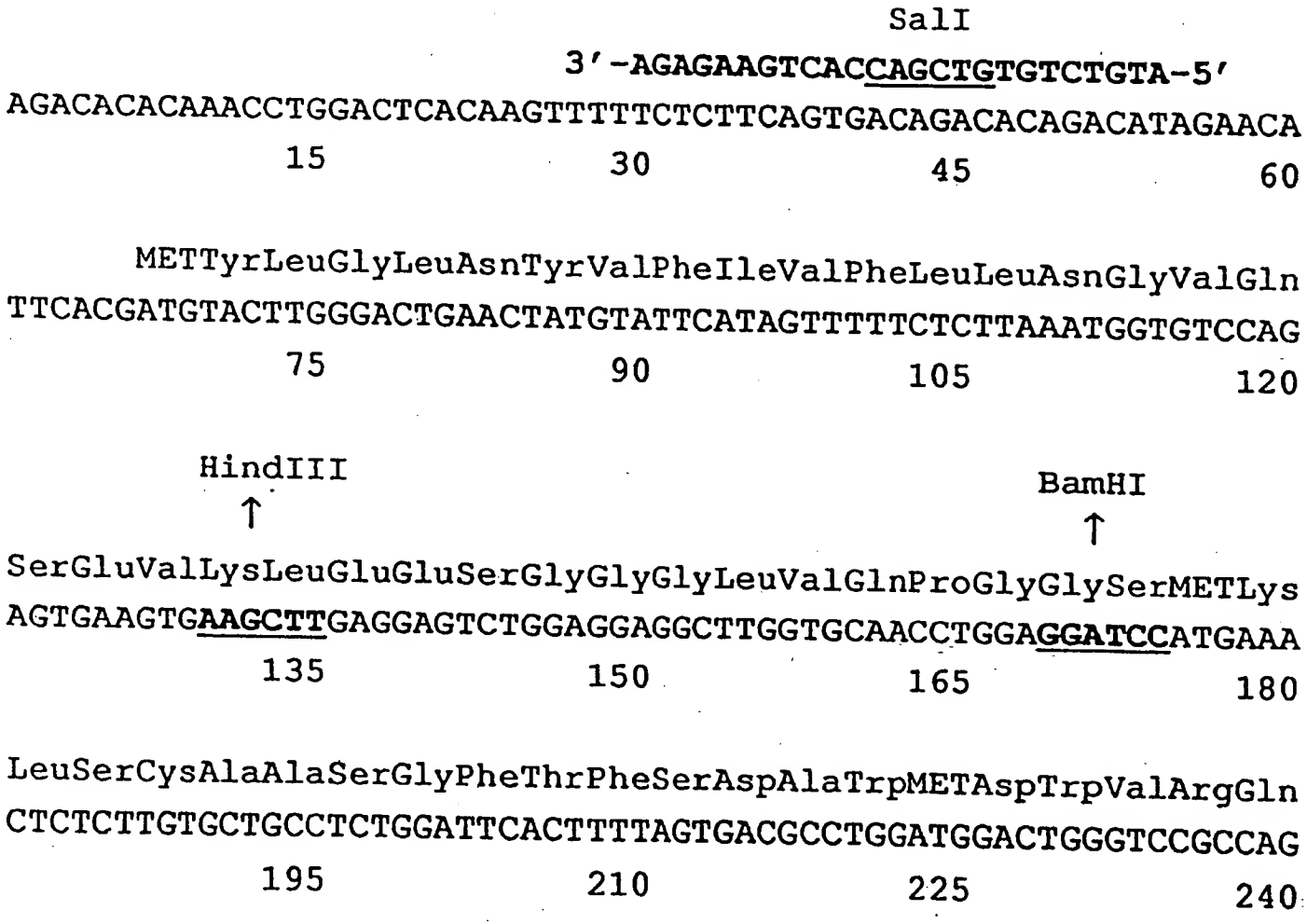
SalI
3'-AGAGAAGTCACCAGCTGTGTCTGTA-5'
AGACACACAAACCTGGACTCACAAGTTTTTCTCTTCAGTGACAGACACAGACATAGAACA
15
30
45
60
METTyrLeuGlyLeuAsnTyrValPheIleValPheLeuLeuAsnGlyValGln
TTCACGATGTACTTGGGACTGAACTATGTATTCATAGTTTTTCTCTTAAATGGTGTCCAG
75
90
105
120
HindIII
BamHI
SerGluValLysLeuGluGluSerGlyGlyGlyLeuValGlnProGlyGlySerMETLys
AGTGAAGTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAA
135
150
165
180
LeuSerCysAlaAlaSerGlyPheThrPheSerAspAlaTrpMETAspTrpValArgGln
CTCTCTTGTGCTGCCTCTGGATTCACTTTTAGTGACGCCTGGATGGACTGGGTCCGCCAG
195
210
225
240

FIG. 12

```
SerProGluLysGlyLeuGluTrpValAlaGluIleArgSerLysAlaAsnAsnHisAla
TCTCCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGAAGCAAAGCTAATAATCATGCA
          255                 270                 285                 300

ThrTyrTyrAlaGluSerValLysGlyArgPheThrIleSerArgAspAspSerLysSer
ACATACTATGCTGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAAAAGT
          315                 330                 345                 360

SerValTyrLeuGlnMETAsnSerLeuArgAlaGluAspThrGlyIleTyrTyrCysThr
AGTGTCTACCTGCAAATGAACAGCTTAAGAGCTGAAGACACTGGCATTTATTACTGTACC
          375                 390                 405                 420

                                                PstI
                                                 ↑
AspTrpPheAlaTyrTrpGlyGlnGlyThrLeuValThrValSerAla
GACTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAG
          435                 450                 465
```

FIG. 12 (cont.)

SalI

↑ METMETSerSerAlaGlnPheLeuGlyLeuLeuLeuLeu

CAAGTCAAGACTCACCTGGACATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTC

15 30 45 60

PstI

3′-AAGTTCCATGGTGACGTCTATAGGTCTAC-5′

CysPheGlnGlyThrArgCysAspIleGlnMETThrGlnThrThrSerSerLeuSerAla

TGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCC

75 90 105 120

SerLeuGlyAspArgValThrIleSerCysSerAlaSerGlnGlyIleSerAsnTyrLeu

TCTCTGGGAGACAGAGTCACCATCAGTTGCAGTGCAAGTCAGGGCATTAGCAATTATTTA

135 150 165 180

FIG. 13

AsnTrpTyrGlnGlnLysProAspGlyThrValLysLeuLeuIleTyrTyrThrSerSer
AACTGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTATTACACATCAAGT
195 210 225 240

LeuHisSerGlyValProSerArgPheSerGlySerGlySerGlyThrAspTyrSerLeu
TTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGGACAGATTATTCTCTC
255 270 285 300

HindIII
ThrIleSerAsnLeuGluProGluAspIleAlaThrTyrTyrCysGlnGlnTyrSerLys
ACCATCAGCAACCTGGAACCTGAAGATATTGCCACTTACTATTGTCAGCAGTATAGTAAG
315 330 345 360

BglII
3′-GGTTCGACCTCTAGATTGCCCGACTAC-5′
LeuProTrpThrPheGlyGlyGlyThrLysLeuGluIleLys
CTTCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAA
375 390

FIG. 13 (cont.)

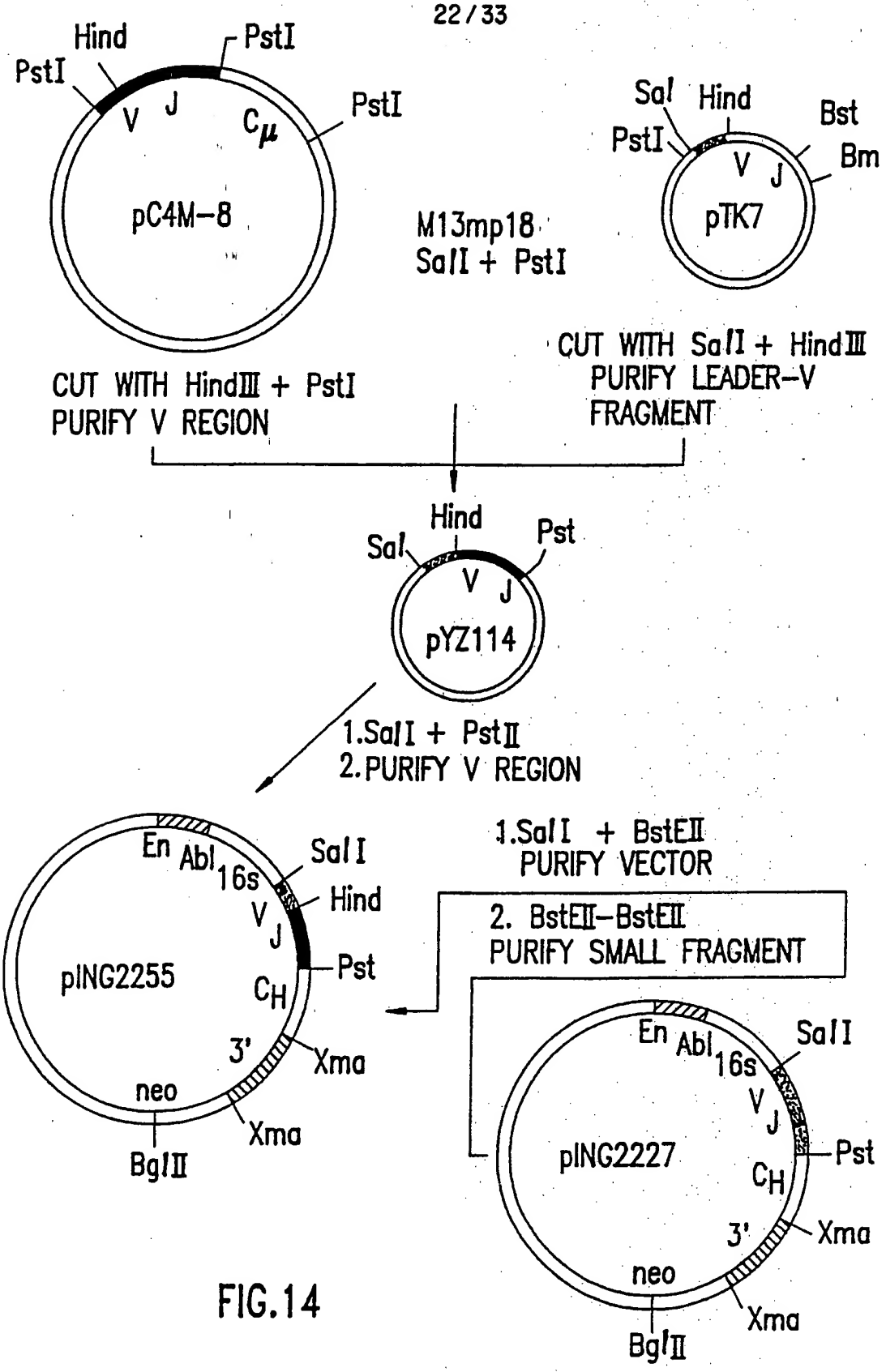
Hind
PstI
PstI
PstI
V
J
Cμ
pC4M-8
M13mp18
SalI + PstI
Sal
Hind
Bst
PstI
Bm
V
J
pTK7
CUT WITH HindIII + PstI
PURIFY V REGION
CUT WITH SalI + HindIII
PURIFY LEADER-V
FRAGMENT
Hind
Sal
Pst
V
J
pYZ114
1.SalI + PstII
2.PURIFY V REGION
1.SalI + BstEII
PURIFY VECTOR
2. BstEII-BstEII
PURIFY SMALL FRAGMENT
En
Abl
16s
Sal I
Hind
V
J
pING2255
CH
Pst
3'
Xma
neo
Xma
BglII
En
Abl
16s
SalI
V
J
pING2227
CH
Pst
3'
Xma
neo
Xma
BglII

FIG.14

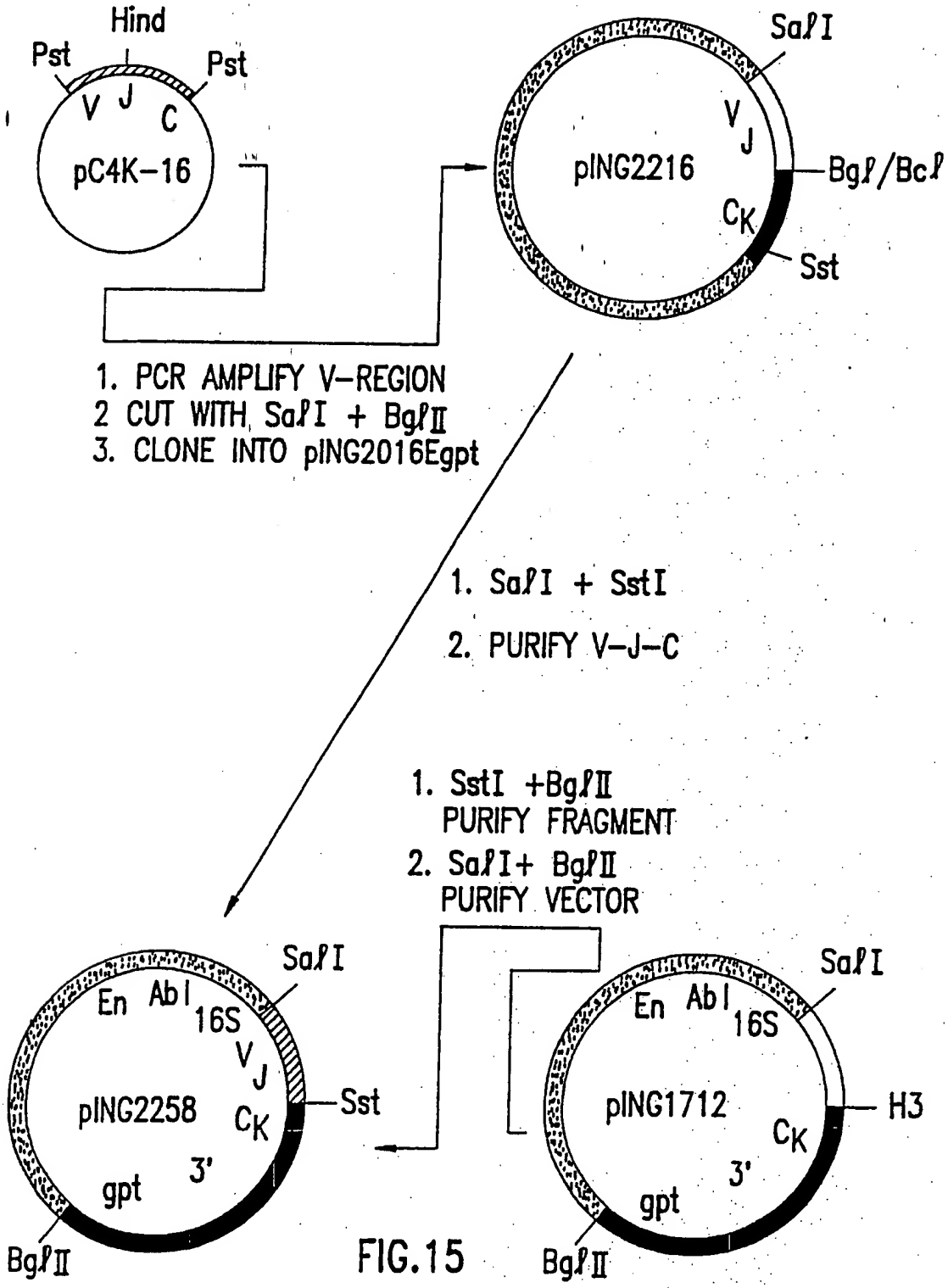
Hind
Pst
Pst
V J C
pC4K-16
SalI
V J
pING2216
BglI/BclI
CK
Sst
1. PCR AMPLIFY V-REGION
2 CUT WITH SalI + BglII
3. CLONE INTO pING2016Egpt
1. SalI + SstI
2. PURIFY V-J-C
1. SstI +BglII
PURIFY FRAGMENT
2. SalI+ BglII
PURIFY VECTOR
SalI
En Abl 16S
V J
pING2258
CK
Sst
gpt
3'
BglII
SalI
En Abl 16S
pING1712
CK
H3
3'
gpt
BglII

FIG.15

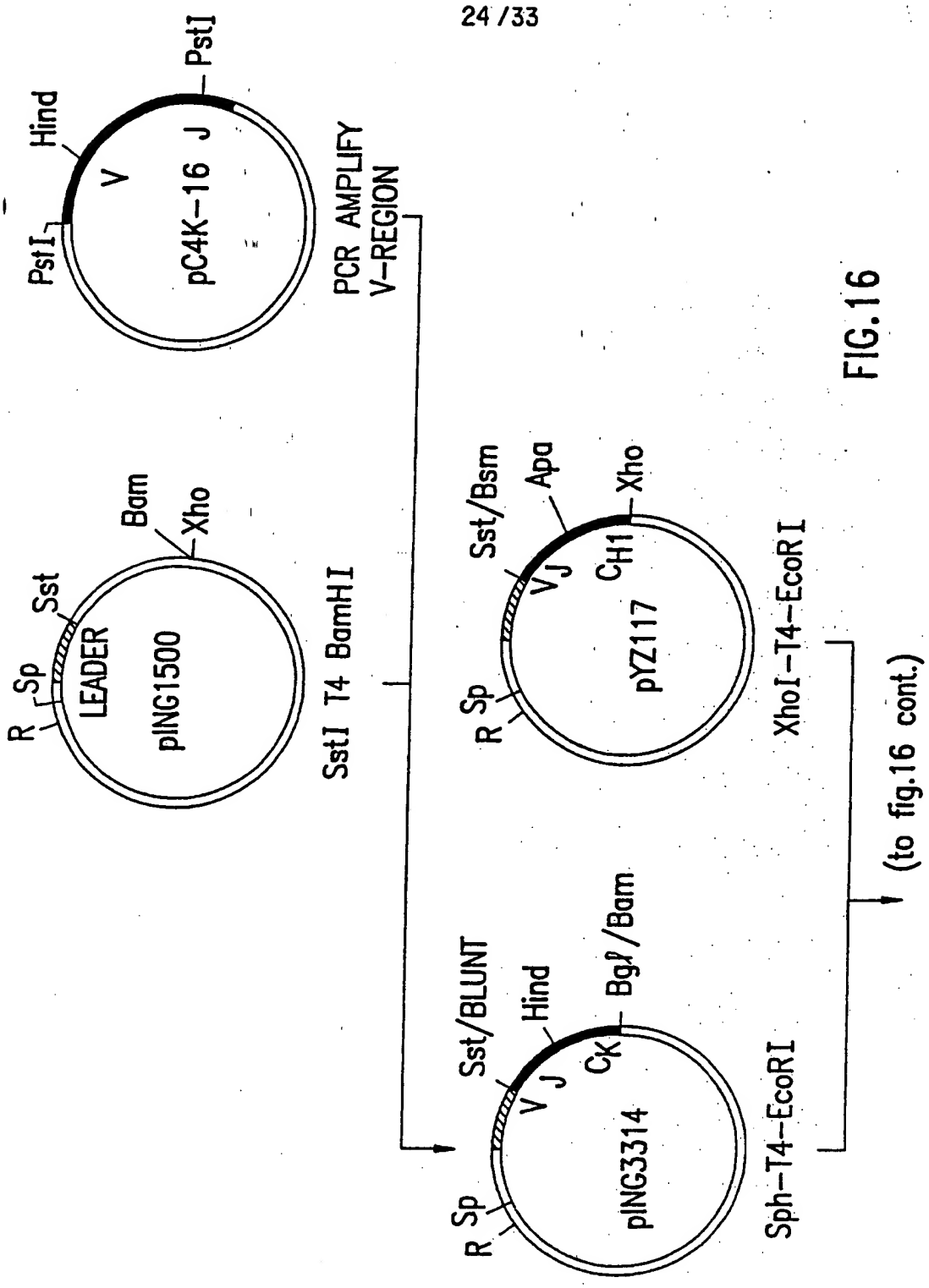
PstI
Hind
V
J
pC4K-16
PstI
PCR AMPLIFY
V-REGION
Bam
Xho
Sst
Sp
LEADER
pING1500
R
SstI T4 BamHI
Sst/Bsm
Apa
Xho
VJ
CH1
pYZ117
R Sp
XhoI-T4-EcoRI
(to fig.16 cont.)
Sst/BLUNT
Hind
Bgl/Bam
VJ
CK
pING3314
R Sp
Sph-T4-EcoRI

FIG.16

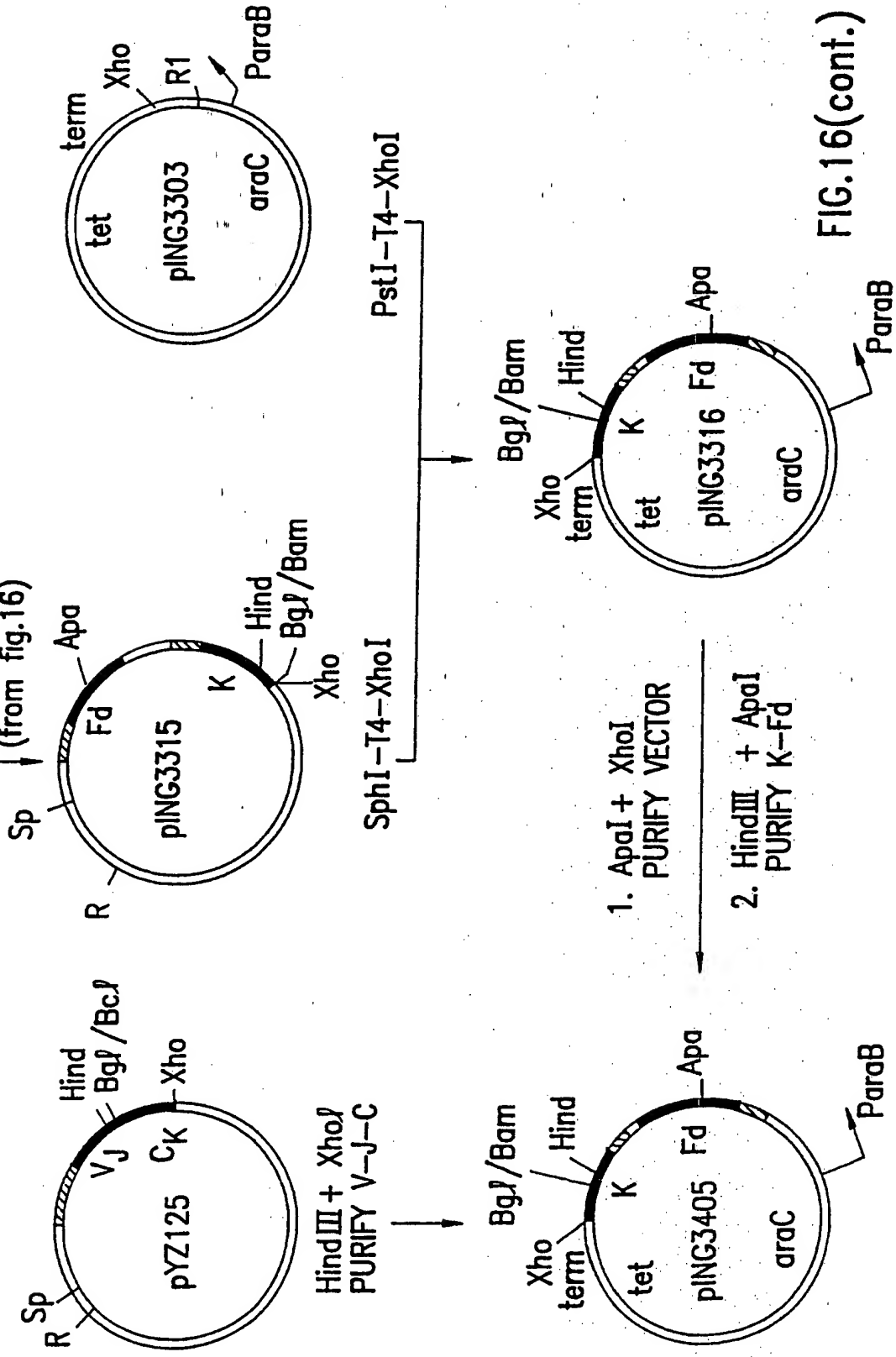
pYZ125
Sp
R
Hind
Bgl/Bc
Xho
VJ
CK
HindIII + XhoI
PURIFY V-J-C
pING3405
tet
term
Xho
Bgl/Bam
Hind
K
Fd
Apa
araC
ParaB
1. ApaI + XhoI
PURIFY VECTOR
2. HindIII + ApaI
PURIFY K-Fd
pING3316
tet
term
Xho
Bgl/Bam
Hind
K
Fd
Apa
araC
ParaB
PstI-T4-XhoI
SphI-T4-XhoI
(from fig.16)
pING3315
Sp
R
Apa
Fd
K
Hind
Bgl/Bam
Xho
pING3303
tet
term
Xho
R1
araC
ParaB

FIG.16(cont.)

```
                BclI
                 ↑                                                    -
                                                                     MET
CCAAACAACCTATGATCAGTGTCCTCTCAACAGTCCCTGAACACACTGACTCTCACCATG
              15                  30                  45                  60

                                                          ApaI
                                                3'-CGTCCACAAGCCCGGGTCCAG
GluTrpSerGlyValPheIlePheLeuLeuSerValThrAlaGlyValHisSerGlnVal
GAGTGGAGCGGAGTCTTTATCTTTCTCCTGTCAGTGACTGCAGGTGTTCACTCCCAGGTC
              75                  90                  105                 120

GT-5'
HisLeuGlnGlnSerGlyAlaGluMETValArgProGlyThrSerValLysValSerCys
CACCTACAGCAGTCTGGAGCTGAGATGGTAAGGCCTGGGACTTCCGTGAAAGTGTCCTGC
              135                 150                 165                 180

ArgAlaSerGlyTyrAlaPheThrAsnTyrLeuIleGluTrpValLysGlnArgProGly
AGGGNTTCTGGATACGCCTTCACTAATTACTTGATAGAGTGGGTTAAGCAGAGGCCTGGA
              195                 210                 225                 240
```

FIG. 17

GlnGlyLeuGluTrpIleGlyValIleAsnProGlySerGlyGlyThrThrTyrAsnGlu
CAGGGCCTTGAGTGGATTGGAGTGATTAATCCTGGAAGTGGTGGTACTACCTACAATGAG
255 270 285 300

LysPheLysAspLysThrThrMETThrAlaAspLysSerSerSerThrAlaTyrMETHis
AAGTTCAAGGACAAGACAACAATGACTGCAGACAAGTCTTCCAGCACTGCCTACATGCAC
315 330 345 360

LeuAspSerLeuThrSerAspAspSerAlaValTyrLeuCysAlaArgThrGlySerGly
CTCGATAGCCTGACATCTGATGACTCTGCGGTTTATCTCTGTGCCAGAACTGGGTCAGGG
375 390 405 420

BstEII
3'-TCCTTGGAGCCAGTGGCAGAG-5'
HisAlaLeuGluTyrTrpGlyGlnGlyThrSerValThrValSerSer
CATGCTTTGGAATACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
435 450 465

FIG. 17 (cont.)

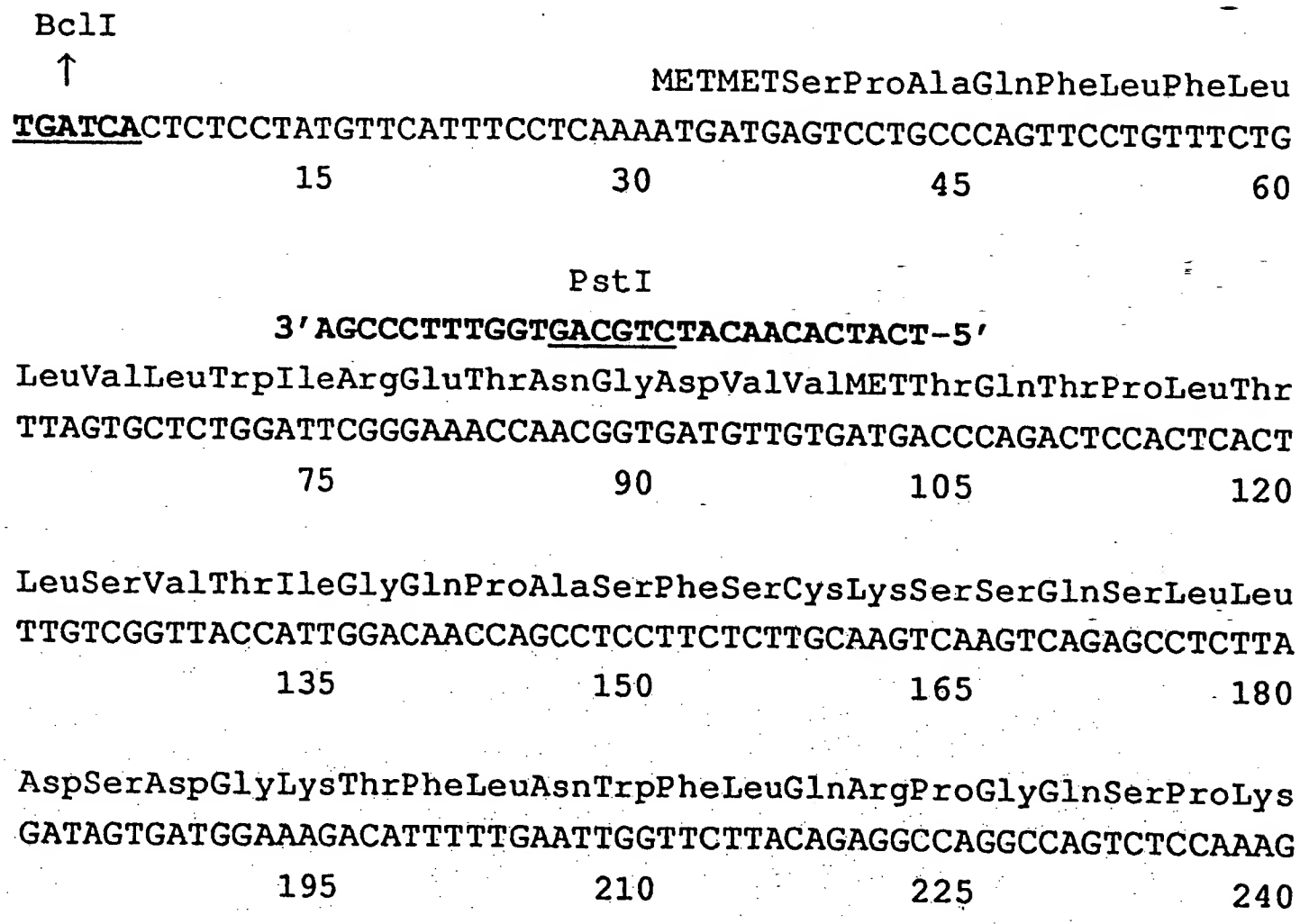
BclI
METMETSerProAlaGlnPheLeuPheLeu
TGATCACTCTCCTATGTTCATTTCCTCAAAATGATGAGTCCTGCCCAGTTCCTGTTTCTG
15 30 45 60
PstI
3'AGCCCTTTGGTGACGTCTACAACACTACT-5'
LeuValLeuTrpIleArgGluThrAsnGlyAspValValMETThrGlnThrProLeuThr
TTAGTGCTCTGGATTCGGGAAACCAACGGTGATGTTGTGATGACCCAGACTCCACTCACT
75 90 105 120
LeuSerValThrIleGlyGlnProAlaSerPheSerCysLysSerSerGlnSerLeuLeu
TTGTCGGTTACCATTGGACAACCAGCCTCCTTCTCTTGCAAGTCAAGTCAGAGCCTCTTA
135 150 165 180
AspSerAspGlyLysThrPheLeuAsnTrpPheLeuGlnArgProGlyGlnSerProLys
GATAGTGATGGAAAGACATTTTTGAATTGGTTCTTACAGAGGCCAGGCCAGTCTCCAAAG
195 210 225 240

FIG. 18

ArgLeuLeuTyrLeuValSerLysLeuAspSerGlyValProAspArgPheThrGlySer
CGCCTACTCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCAGT
255 270 285 300

GlySerGlyThrAspPheThrLeuLysIleSerArgValGluAlaGluAspLeuGlyVal
GGATCAGGGACAGATTTCACACTGAAGATCAGCAGAGTGGAGGCTGAGGATTTGGGAGTT
315 330 345 360

HindIII
3'-CCCTGGTTCGAACTC
TyrTyrCysTrpGlnGlySerHisPheProIleThrPheGlyAlaGlyThrLysLeuGlu
TATTATTGCTGGCAAGGTTCACATTTTCCGATCACGTTCGGTGCTGGGACCAAGCTAGAA
375 390 405 420

GAC-5'
LeuArg
CTGAGA

FIG. 18 (cont.)

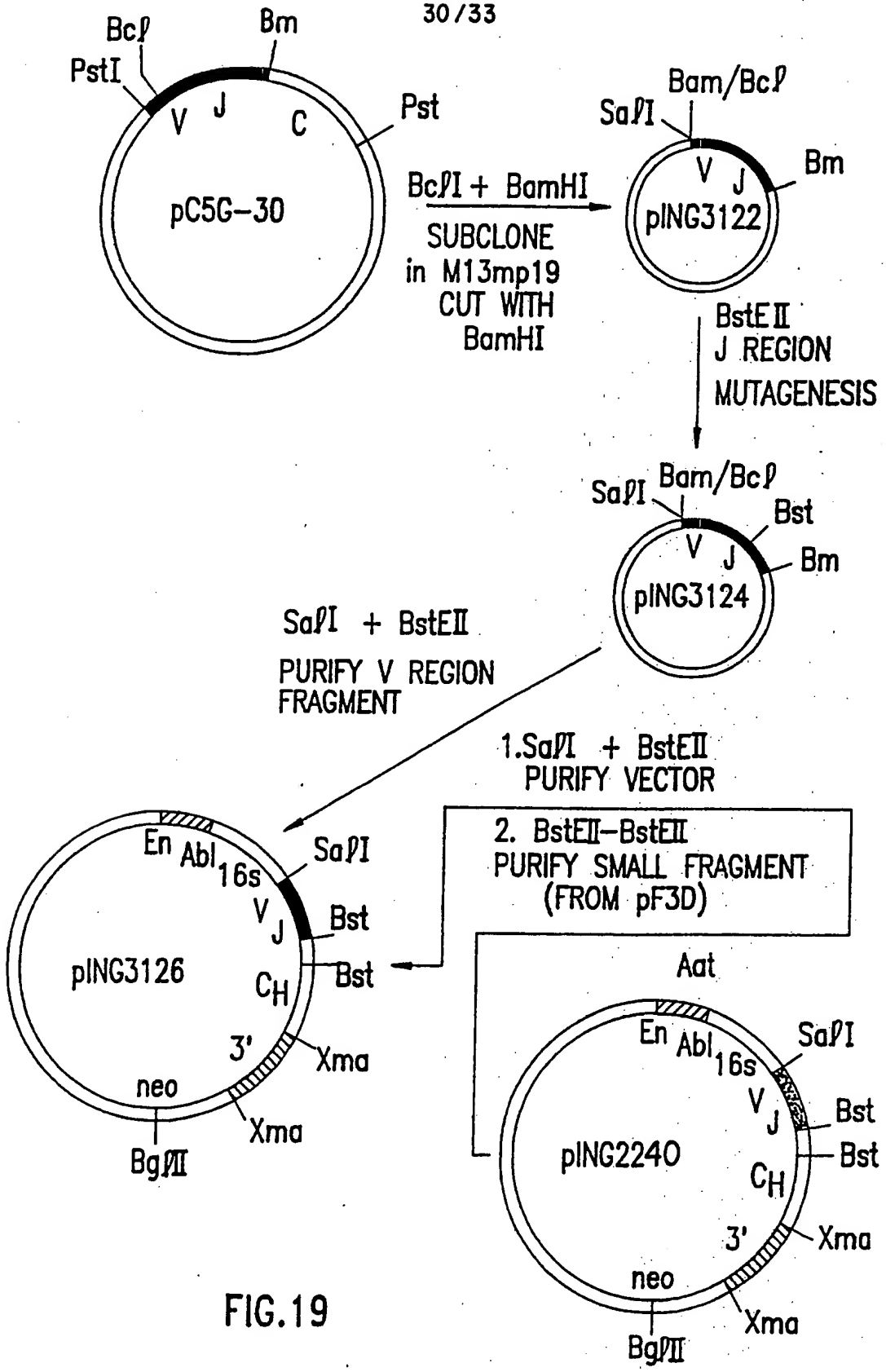
BclI
Bm
PstI
V
J
C
Pst
pC5G-30
BclI + BamHI
SUBCLONE
in M13mp19
CUT WITH
BamHI
Bam/BclI
SalI
V
J
Bm
pING3122
BstEII
J REGION
MUTAGENESIS
SalI
Bam/BclI
Bst
V
J
Bm
pING3124
SalI + BstEII
PURIFY V REGION
FRAGMENT
1.SalI + BstEII
PURIFY VECTOR
2. BstEII–BstEII
PURIFY SMALL FRAGMENT
(FROM pF3D)
En
Abl
16s
SalI
V
J
Bst
pING3126
CH
Bst
3'
Xma
neo
Xma
BglII
Aat
En
Abl
16s
SalI
V
J
Bst
pING2240
CH
Bst
3'
Xma
neo
Xma
BglII

FIG.19

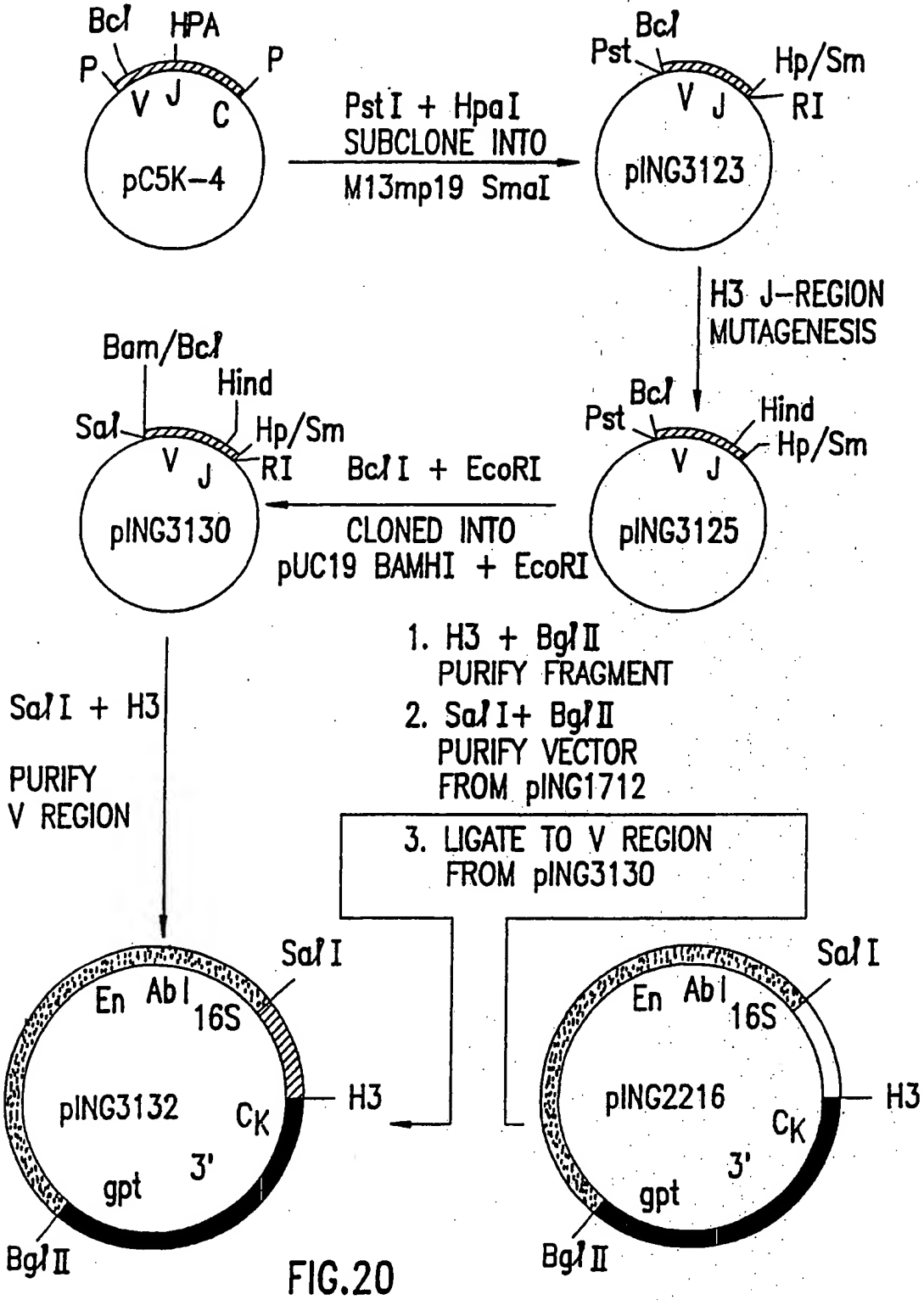
BclI
HPA
P
P
V J C
pC5K-4
PstI + HpaI
SUBCLONE INTO
M13mp19 SmaI
BclI
Pst
Hp/Sm
V J
RI
pING3123
H3 J-REGION
MUTAGENESIS
Bam/BclI
Hind
SalI
Hp/Sm
V J
RI
pING3130
BclI + EcoRI
CLONED INTO
pUC19 BAMHI + EcoRI
BclI
Pst
Hind
Hp/Sm
V J
pING3125
1. H3 + BglII
PURIFY FRAGMENT
2. SalI+ BglII
PURIFY VECTOR
FROM pING1712
SalI + H3
PURIFY
V REGION
3. LIGATE TO V REGION
FROM pING3130
SalI
En Ab I
16S
pING3132
CK
H3
3'
gpt
BglII
SalI
En Ab I
16S
pING2216
CK
H3
3'
gpt
BglII

FIG.20

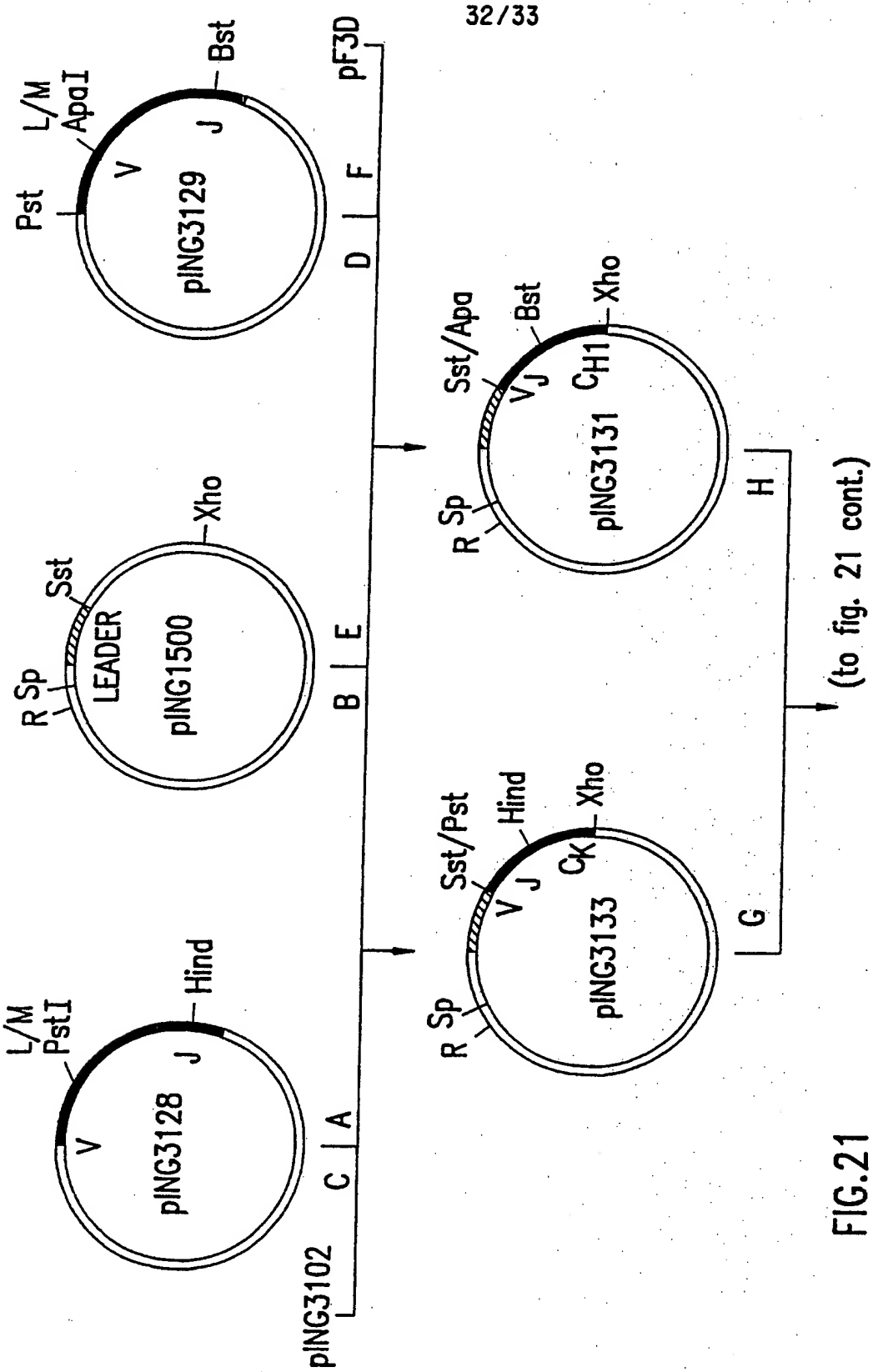
pING3102
C
A
L/M
PstI
V
pING3128
J
Hind
B
E
R Sp
LEADER
Sst
pING1500
Xho
D
F
pF3D
Pst
L/M
ApaI
V
pING3129
J
Bst
R Sp
Sst/Apa
VJ
pING3131
CH1
Bst
Xho
H
G
R Sp
Sst/Pst
VJ
pING3133
CK
Hind
Xho
(to fig. 21 cont.)

FIG.21

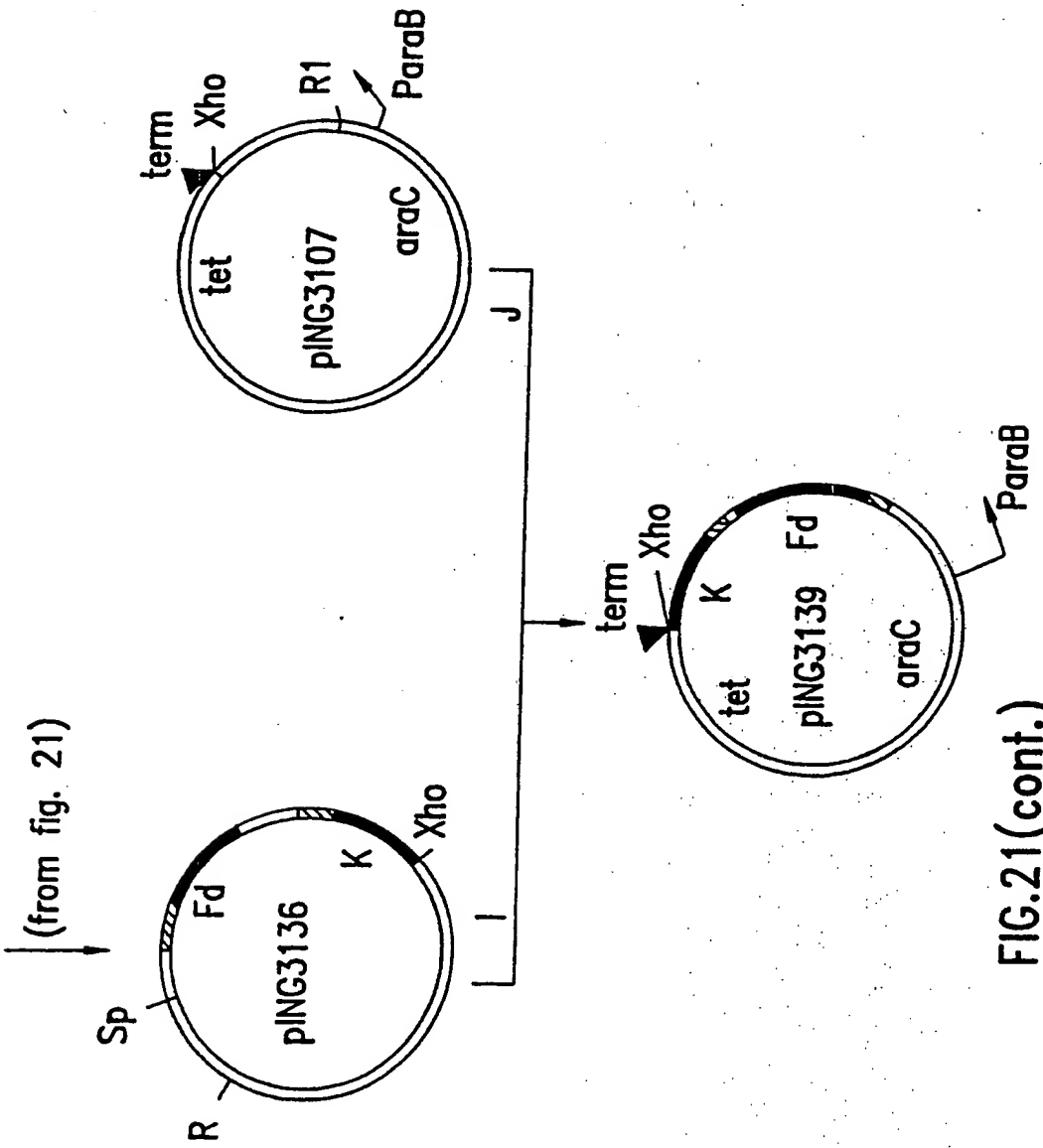
(from fig. 21)
Sp
R
pING3136
Fd
K
Xho
I
J
term
Xho
R1
ParaB
tet
pING3107
araC
term
Xho
K
Fd
ParaB
tet
pING3139
araC

FIG.21(cont.)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/06627

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12N 15/13
U.S.Cl.: 424/85.8

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/69.1, 7, 70.21; 935/81; 424/85.8, 85.91; 536/27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DIALOG: FILES (155, 351), USPTO Automated Patent System (FILE USPAT, 1975-1990). See Attachment for terms searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Proceedings of the National Academy of Sciences (Washington, USA), Vol. 84 Issued January 1987, Sun et al, "Chimeric antibody with human constant regions and mouse variable regions directed against carcinoma associated antigen 17-1A", pages 214-218, see entire document.	1-11
X	Proceedings of the National Academy of Sciences (Washington, USA), vol. 85, issued June 1988, Leahy et al, "Sequences of 12 monoclonal anti-dinitro-phenylspin-label antibodies for NMR studies", pages 3661-3665, see entire document.	1-11

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 February 1991	21 MAR 1991
International Searching Authority	Signature of Authorized Officer
ISA/US	Thomas Nisbet

Form PCT/ISA/210 (second sheet) (rev 11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	The Journal of Biological Chemistry (Baltimore, USA), vol. 262, issued 05 October 1987, Chen et al, "Nucleotide and Translated Amino Acid Sequences of the Light and Heavy Chains of Mouse Hybridoma Antibodies to Blood Group A and B Substances", pages 13579-13583, see entire document.	1-11
X	WO,A, 89/05820 (Gurney) 21 December 1987, see entire document.	1-11
X	WO,A, 88/09181 (Chang et al) 29 May 1987, see entire document.	1-11
X	Molecular Immunology, vol. 25, issued 1988, (New York, USA) Kaartinnen et al Combinatorial association of κ genes: One V- genecodes for 3 non-cross-reactive monoclonal antibodies each specific for a different antigen, pages 859-865, see entire document	1-11
X	Journal of Clinical Investigation, vol. 82, issued 1988, (New York, USA), Kofler et al, "Immunoglobulin Kappa light chain variable region gene complex organization and immunoglobulin genes encoding anti-DNA autoantibodies in lupus mice", pages 852-860, see entire document.	1-11
X	European Journal of Biochemistry, vol. 176, issued 1988 , (Berlin Germany), P. DeWaele et al. "Expression in non-lymphoid cells of mouse recombinant immunoglobulin directed against tumor marker human placental alkaline phosphatase", pages 287-298, see entire document.	1-11

Attachment to form PCT/ISA/210
Continuation in part II
Fields Searched

Search terms

antibody	p 160
immunoglobulin	p 120
ig?	p 55
HIV	p 45
AIDS	p 39
HTLV	p 24
	p 18
	p 65
	p 51

PIR sequence search of all sequences was also performed

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